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(54) Title: IMMUNOCONJUGATES COMPRISING SINGLE-CHAIN VARIABLE REGION FRAGMENTS OF ANTI-CD-19 ANTIBODIES

(57) Abstract

Disclosed are polynucleotides encoding single chain variable region fragments of a monoclonal antibody to CD19 and methods for preparing the same. Also disclosed are single chain variable region polypeptides, methods for preparing the same, point modified polypeptides, and dimers derived therefrom. An additional aspect of the invention discloses immunoconjugates formed between a polypeptide of the invention and cytotoxic agents, as well as methods for their preparation, as well as use in the treatment of cancer.

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IMMUNOCONJUGATES COMPRISING SINGLE-CHAIN VARIABLE REGION FRAGMENTS OF ANTI-CD-19 ANTIBODIES

POSSIBLE GOVERNMENT OWNERSHIP RIGHTS

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BACKGROUND OF THE INVENTION

Immunoconjugates

Antibodies directed against cell surface molecules defined by cluster differentiation (CD) antigens represent a unique opportunity for the development of therapeutic reagents. Certain CD antigen expression is highly restricted to specific lineage lymphohematopoietic cells and, over the past several years, antibodies directed against lymphoid-specific CD antigens have been used to develop treatments that were effective either *in vitro* or in animal models (Ghetie *et al.*, 1988; Uckun *et al.*, 1986; Myers *et al.*, 1991; Jansen *et al.*, 1992). However, due to their large size, intact antibodies and antibody-toxin conjugates have several disadvantages that limit their efficiency. They are restricted in their ability to migrate from

the vascular system, are heterogeneous as immunoconjugates (which can result in linkage of several toxin molecules to one immunoglobulin molecule), and their production is expensive and very labor intensive. See, for example, U.S. Patent No. 4,831,117 to Uckun and U.S. Patent No. 4,671,958 to Rodwell, *et al.*, the teachings of which are herein incorporated specifically by reference.

The limited efficacy of many unmodified monoclonal antibodies has led to an alternative approach, the use of these agents as carriers of cytotoxic substances. An array of toxins of bacterial and plant origin have been coupled to monoclonal antibodies for production of immunotoxins (Schlom; Pastan *et al.*, 1986). The strategy is to select from nature a toxic protein and then to modify the toxin so that it will no longer indiscriminately bind and kill normal cells but will instead kill only the cells expressing the antigen identified by the monoclonal antibody. The majority of toxins targeted to cell surfaces by immunoconjugates act in the cytoplasm, where they inhibit protein synthesis. After binding to cell surface antigens, immunotoxins are taken up by endocytosis and delivered to endosomes. Fragments of some toxins (for example, diphtheria toxin) are then translocated across the membrane of this organelle. Other immunotoxins (for example,ricin) are routed further to the trans-Golgi network, where a minority undergo translocation to the cytoplasm. Unfortunately, most are routed to lysosomes, where they are degraded. In the cytoplasm, the toxins used clinically act either to adenosine diphosphate (ADP)-ribosylate elongation factor 2 (for example, *Pseudomonas* exotoxin (PE)) or to inactivate the 60S ribosomal subunit so that it has a decreased capacity to bind elongation factor 2 (for example, ricin). Less than ten toxin molecules in the cytoplasm are sufficient to kill the cell; however, more must bind to the cell surface to compensate for the inefficiencies in internalization and translocation.

Although immunotoxins are simple in concept, the first-generation immunotoxins were relatively ineffective. Several requirements must be fulfilled for an immunotoxin to be effective (Pastan *et al.*, 1986). In

particular: (i) the immunoconjugate should be specific and should not react with normal tissues. Binding to tissues that do not express antigen can be reduced by removal of the nonspecific natural cell-binding subunits or domains of the toxin. Furthermore, because plant glycoprotein toxins contain mannose oligosaccharides that bind to cells of the reticuleondothelial system and, in some cases, also contain fucose residues that are recognized by the receptors on hepatocytes, deglycosylation of plant toxins may be required to avoid rapid clearance and potential cytotoxic effects on these cells. (ii) The linkage of the toxin to the antibody should not impair the capacity of the antibody to bind antigen. (iii) The immunotoxin must be internalized into endosomic vesicles. Thus, toxins directed by monoclonal antibodies to surface receptors that are normally internalized may be more active than those directed toward noninternalizing cell surface molecules. (iv) The active component of the toxin must translocate into the cytoplasm. These various goals can be in conflict; thus, the removal of the B chain of ricin reduces nonspecific binding but also reduces the capacity of the residual A-chain monoclonal antibody conjugate to translocate across the endosomic vesicle membrane. (v) For *in vivo* therapy, the linkage must be sufficiently stable to remain intact while the immunotoxin passes through the tissues of the patient to its cellular site of action. The first generation of heterobifunctional cross-linkers used to bind the toxin to the monoclonal antibody generated disulfide bonds that were unstable *in vivo*. This problem was solved in part by the synthesis of more stable cross-linkers, which used phenyl or methyl groups, or both, adjacent to the disulfide bond to restrict access to the bond.

The activity of an immunotoxin is initially assessed by measuring its ability to kill cells with target antigens on their surfaces. Because toxins act within the cells, receptors and other surface proteins that naturally enter cells by endocytosis usually make good targets for immunotoxins, but surface proteins that are fixed on the cell surface do not. However, if several antibodies recognizing different epitopes on the same cell surface

protein are available, it is useful to test them all, because some, perhaps by producing a conformational change in the target protein's structure, may induce its internalization or direct its intracellular routing to an appropriate location for toxin translocation (May *et al.*, 1991; Press *et al.*, 1988). Also, it is possible to induce internalization of a target surface protein if the immunotoxin contains a form of PE or ricin in which the binding of the toxin moiety to its receptor, although weakened by chemical modification, still occurs and promotes internalization since toxin receptors are efficiently internalized (Willingham *et al.*, 1987; Lambert *et al.*, 1991; Colombatti *et al.*, 1986).

Several immunotoxins have been developed and approved for human trials. Two different kinds of trials have been conducted. The first involves the *ex vivo* addition of immunotoxins to harvested bone marrow to eliminate contaminating tumor cells before reinfusion in patients undergoing autologous bone marrow transplantation. A variety of antibodies, linked to ricin or ricin A chain, including anti-CD5 and anti-CD7, have been used for this purpose (Uckun *et al.*, 1990b). The second kind of trial involves the parenteral administration of immunotoxins, either regionally (such as the peritoneal cavity) or systematically, to patients with cancer. These have been primarily Phase 1 and 2 trials in patients in which conventional treatments have failed, and the patients have a large tumor burden. So far, the antibodies used for the preparation of immunotoxins to treat carcinomas or other solid tumors have been found to react with important normal human tissues (such as neural tissue and bone marrow) and produce dose-limiting toxicity without significant clinical responses (Weiner *et al.*, 1989; Gould *et al.*, 1989; Byers *et al.*, 1989; Pai, *in press*).

Cell Differentiation Antigens

The maturation of human BCPs into functional B lymphocytes represents a developmentally programmed multi-step process, which is accompanied by a cascade of somatic immunoglobulin gene rearrangements (Korsmeyer *et al.*, 1981), as well as a coordinated

acquisition and loss of B-lineage differentiation antigens (Nadler). The characterization and classification of these antigens have been standardized during the first (Paris, France, 1982), second (Boston, MA, 1984), third (Oxford, UK, 1986), and fourth (Vienna, Austria, 1989) International Workshops on Human Leukocyte Differentiation Antigens, and a World Health Organization (WHO)-established CD (cluster of differentiation) nomenclature has been introduced for their identification (Nadler; Knapp *et al.*, 1989a; Clark *et al.*, 1989).

To date, more than 20 biochemically distinct differentiation antigens have been identified on B-lineage cells not including the surface immunoglobulins (sIg), major histocompatibility (MHC) antigens, or the receptor proteins for defined cytokines. Many of the B-lineage differentiation antigens represent functionally important surface receptors on developing B-lineage cells, and their expression is regulated by different external signals (Knapp *et al.*, 1989a; Clark *et al.*, 1989; Zola, 1987). While some (such as CD10, CD45, and CD73) represent membrane-associated enzymes, others (such as CD19, CD22, and B7) likely represent physiologically important cell surface bound ligands, which may play an important role in cell-to-cell interactions during B-cell development in a bone marrow microenvironment (Knapp *et al.*, 1989a; Clark *et al.*, 1989; Zola, 1987). The latter possibility is precedented by published evidence showing that many T-lineage differentiation antigens including CD2, CD4, CD8, and CD18/LFA-1 function as cell-surface bound ligands (CD2 for LFA-3, CD4 for class II MGC, CD8 for class I MHC, CD18/LFA-1 for I-CAM-1/gp80). The heterophilic recognition between such surface receptors may be important for cognate surface interactions between B-lineage cells and T cells or accessory cell populations in lymphohematopoietic tissues. Other B-lineage antigens (such as CD23 and CD40) might function as surface receptors for as yet undefined soluble cytokines (Clark *et al.*, 1989).

CD19, CD22, and B7 antigens are members of the Ig supergene family (Knapp *et al.*, 1989b; Stamenkovic, 1988; Stamenkovic, 1990; Freeman *et al.*, 1989). CD21 has been identified as the C3d receptor as well

as a receptor for Epstein-Barr virus (EBV) (Knapp *et al.*, 1989b). The cytoplasmic domain of CD19 shows homology to proteins encoded by the int-1 oncogene and by EBV (Stamenkovic, 1988). CD19 has been proposed as a bridging molecule important for transduction of sIg-mediated signals in mature B cells (Pesando *et al.*, 1989; Carter *et al.*, 1990). CD19 as a signal-transducing subunit and CD21 as a ligand-binding subunit linking the B cell to the complement system have been reported to form a complex on the surface of B cells which may be involved in the sIg-dependent activation. However, the function of the CD19 molecule is not dependent on the presence of sIg or CD21 because CD19 ligation results in stimulation of phosphoinositide turnover (Uckun *et al.*, 1989) and calcium mobilization in sIg-CD21-BCP populations and modulates their proliferative activity (Uckun *et al.*, 1988; Ledbetter *et al.*, 1988). CD22 displays a high degree of homology to the myelin-associated glycoprotein (MAG), a neuronal surface adhesion molecule mediating cell-to-cell interactions between B cells and monocytes (Stamenkovic, 1990. Furthermore, CD22 may also be important for transduction of sIg-mediated signals (Pezzutto *et al.*, 1988). Most recently, the natural ligand of B7 antigen has been identified as the CD28 T-cell activation antigen, which is another member of the Ig superfamily (Linsley *et al.*, 1990). CD28-B7 mediated adhesion between activated B cells and T cells might be important for T-cell regulation of antigen-specific B-cell responses.

Monoclonal Antibodies and Fragments

Monoclonal antibodies have largely been applied clinically to the diagnosis and therapy of cancer and the modulation of the immune response to produce immunosuppression for treatment of autoimmune and graft versus host diseases (GVHD) and for prevention of allograft rejection. Human monoclonal antibodies have also been applied clinically against cytomegalovirus, *Varicella zoster* virus, and the various specific serotypes of *Pseudomonas aeruginosa, Escherichia coli,* and *Klebsiella pneumoniae.*

Antibodies or their fragments can also be genetically engineered to

have more rapid clearance. This is desirable when a monoclonal antibody is conjugated to a radionuclide for use in radioimmunoscanning. For example, antigen-binding fragment (Fab), $F(ab')_2$, or single chain Fv fragments of monoclonal antibodies have survival half-lives of less than 5 hours. Rapid turnover can also be accomplished by the deletion of the CH2 domain as demonstrated for an antibody reactive with the disaloganglioside GD2 expressed on human tumors of neuroectodermal origin (Müeller *et al.*, 1990).

In an attempt to improve on the efficacy of anti-tumor cytotoxicity of antibodies and immunoconjugates, several laboratories have developed strategies for the expression of the light and heavy chain variable regions of antibodies in bacteria as single chain Fv (scFv) fragments (Pastan *et al.*, 1991; Huston *et al.*, 1988). In general, these molecules have been insoluble and need to be denatured and refolded before binding activity can be detected. One problem with production of antibody binding domains in this manner is that high affinity antibody binding cannot be successfully reconstituted in all instances. The parameters that govern the ability of an antibody to yield an scFv that can bind its target are unknown, thus necessitating the direct cloning and analysis of the candidate antibody gene segments.

The CD19 antigen, which is found on mature B cells but not on plasma cells, has proven to be a very useful target for development of immunoconjugates because most lymphomas and B lineage leukemias express this differentiation marker (Uckun *et al.*, 1990a). Anti-CD19 immunoconjugates have relied on the chemical conjugation of the antibody and a modified catalytic toxin such as the A chain of ricin (Ghetie *et al.*, 1988) or pokeweed antiviral protein (Uckun *et al.*, 1986; Myers *et al.*, 1991). Prior to the development of the present invention, there have been no reports of the development of a successful scFv directed against the CD19 antigen.

The ability of immunotoxins to kill specific subsets of cells efficiently *in vitro* has led to their application in the deletion of particular

cell types in suspensions of bone marrow cells (Thorpe *et al.*, 1982; Seon, 1984; Vallera *et al.*, 1982; Filipovich *et al.*, 1984; Vallera *et al.*, 1983; Muirhead *et al.*, 1983; Krolick *et al.*, 1982). The ultimate objective is to facilitate bone marrow transplantation in the human as an approach to treatment of cancer and diseases of the hematopoietic system. Autologous bone marrow transplantation is used as an adjunct to treatment for certain types of cancer which are highly susceptible to X-irradiation and or chemotherapy (Thomas, 1982; Raso, 1982). The approach is to obtain bone marrow from a patient in remission (preferably in the first remission) and to freeze it. If the patient subsequently relapses, the patient is then subjected to "supralethal" therapy with X-irradiation and or chemotherapy in order to eradicate the tumor. The patient is then rescued from death by infusion of his own bone marrow.

It would, of course, be highly desirable to purge such bone marrow of cancer cells by a cancer cell-reactive immunotoxin. The only requirement of such an immunotoxin is that it should not damage the stem cells which are needed to reconstitute the patient's hematopoietic system.

Immunoconjugates may be utilized for *ex vivo* purging of neoplastic cells from patient bone marrow grafts. These autologous grafts are reintroduced into leukemic patients after aggressive supra lethal chemotherapy and irradiation. The objective of all strategies is to deplete neoplastic cells while leaving unharmed the pluripotent hematopoietic stem cells which repopulate the patient's marrow after reinfusion. Intact immunoconjugates selectively eliminate antigen-positive targets without endangering engraftment and without causing intoxication.

Autologous marrow may be purged of residual leukemia cells without destroying hematopoietic stem cells by the use of immunoconjugates either *in vivo* or *ex vivo*. *Ex vivo* treatment with immunoconjugates has been shown to eliminate most T or B cells present in human marrow without damaging the ability of the marrow to reconstitute lethally irradiated recipients. While the efficiency of

immunoconjugates to kill "the last" leukemic cells still remains an issue the even greater efficiency of radiolabeled immunoconjugates should greatly increase the chances of successful treatment.

Radiolabeled Immunoconjugates

It has been reported that an immunotoxin can specifically eliminate more than 99.99% of clonogenic leukemic T cells even in the presence of excess human bone marrow. The use of a radiolabeled immunotoxin should eliminate even more leukemic T cells, possibly at a rate of greater than 5 logs or 99.999%, indicating that the radiolabeled immunotoxin may be extremely useful for the *ex vivo* elimination of leukemic cells in autologous BMT.

Radiolabeled monoclonal antibodies have been developed as alternative immunoconjugates for delivery of a cytotoxic effector to target cells and for radioimaging (Schlom; Kozak *et al.*, 1985). These species possess potential to compensate for the observed shortcomings of immunotoxins. Toxin conjugates do not pass easily from the endosome to the cytosol. Furthermore, the toxins are immunogenic and thus provide only a short therapeutic window before the development of antibodies directed toward the toxin.

Radioimmunodetection with the use of radiolabeled monoclonal antibodies, most often with monoclonal antibodies to carcinoembryonic antigen, is widely used to complement other approaches for tumor detection. Although intact IgG antibodies are retained better by tumors and thus appear to be better for therapy, $F(ab')_2$ and Fab fragments are preferred for imaging because both targeting and blood clearance are most rapid, which reduces the background. Tumors as small as 0.5 cm, which are sometimes missed by other radiological methods, can be imaged with antibodies or antibody fragments labeled with suitable radionuclides.

One advantage in the use of radiolabeled monoclonal antibody conjugates for therapy is that with the appropriate choice of radionuclide, radiolabeled monoclonal antibodies can kill cells from a distance of several cell diameters and may therefore kill antigen-negative cells adjacent to

antigen-expressing cells. Furthermore, the radiolabeled antibody need not be internalized to kill the tumor cell. Such techniques are exemplified in the teachings of U.S. Patent No. 4,831,122 to Buschbaum *et al.*, incorporated herein by reference.

In a radiolabeled monoclonal antibody, the radionuclide must be tightly linked to the antibody either directly or by a bifunctional chelate. For a monoclonal antibody-chelate complex to be effective, it must meet criteria in addition to those that are true for all monoclonal antibodies: (i) the chelating agent coupled to the monoclonal antibody should not compromise antibody specificity; (ii) the chelation and radiolabeling procedure should not alter the distribution and catabolism of the monoclonal antibody; and (iii) the bifunctional chelate should not permit elution and thus premature release of the radiolabeled metal *in vivo.* Failure to fulfill this last requirement has led to unacceptable toxicity and reduced efficacy. There are a number of suitable ∞-, ß-, and *y*-emitting radionuclides. Isotopes emitting ß particles, although superior to *y*-emitting radionuclides, are not optimal because their low linear energy transfer released over a relatively long distance results in inefficient local killing of target cells coupled with toxicity to distant normal tissues.

Nevertheless, ß-emitting radionuclides such as ^{131}I, ^{90}Y, ^{188}Re, and ^{67}Cu have been useful in immunotherapy. For example, hepatoma-bearing patients have been successfully treated with ^{131}I-labeled antibodies to ferritin (Order, 1985). Furthermore, ^{90}Y-labeled antibodies to ferritin combined with autologous marrow transplantation resulted in complete remissions in four of eight patients with Hodgkin's disease (Order, 1985). ^{90}Y-labeled anti-Tac was effective in prolonging the survival of cardiac allografts and xenografts in a subhuman primate model (Kozak *et al.*, 1989). In a subsequent trial, ^{90}Y-labeled anti-Tac was evaluated for the treatment of patients with HTLV-I-associated, Tac-expressing ATL. At the doses used (5 and 10 miCi per patient), no toxicity was observed in five of six patients studied; modest granulocytopenia and thrombocytopenia were observed in one patient. Five of these six patients underwent a sustained

partial or complete remission after ^{90}Y-labeled anti-Tac therapy.

The target CD19 antigen, a 95 kDa B lineage restricted phosphoglycoprotein, is not expressed on life-maintaining non-hematopoietic tissues, normal hematopoietic progenitor cells, or most immature normal B-lineage lymphoid progenitor cells, but it is expressed by virtually 100% of B lineage ALLs.

SUMMARY OF THE INVENTION

In a first aspect, the present invention provides an isolated and purified polynucleotide encoding a single chain variable region polypeptide that binds to a CD19 antigen. Preferably, the isolated and purified polynucleotide of the invention encodes a polypeptide that has a molecular weight of approximately 28 kDa. More preferably, the polynucleotide of the invention encodes a polypeptide that binds to a CD19 antigen with a K_a of at least 1×10^9 M^{-1}.

Also provided by the invention is a process for preparing an isolated and purified polynucleotide encoding a single chain variable region polypeptide that binds to a CD19 antigen comprising the steps of (a) isolating RNA from hybridomas producing monoclonal antibodies to CD19 antigen; (b) transcribing isolated RNA to cDNA; (c) amplifying separate cDNA sequences encoding a heavy and a light variable region of the monoclonal antibody by a polymerase chain reaction; (d) cloning of separate amplification products encoding the heavy and the light variable regions, respectively, into vector constructs; (e) cloning of DNA encoding the heavy and the light variable regions, wherein the separate sequences are joined through a linker nucleotide sequence, into a vector construct; and (f) digesting the clones with appropriate restriction endonucleases. Preferably, the process of the invention utilizes a linker sequence that is the polynucleotide sequence described by SEQ ID NO:7.

The present invention also contemplates an isolated and purified polynucleotide preparable by a process comprising the steps of (a) isolating RNA from hybridomas producing monoclonal antibodies to CD19 antigen;

(b) transcribing isolated RNA to cDNA; (c) amplifying separate cDNA sequences encoding a heavy and a light variable region of the monoclonal antibody by a polymerase chain reaction; (d) cloning of separate amplification products encoding the heavy and the light variable regions, respectively, into vector constructs; (e) cloning of DNA encoding the heavy and the light variable regions, wherein the separate sequences are joined through a linker nucleotide sequence, into a vector construct; and (f) digesting the clones with appropriate restriction endonucleases.

Alternatively, the present invention provides an isolated and purified polynucleotide prepared by a process comprising the steps of (a) isolating RNA from hybridomas producing monoclonal antibodies to CD19 antigen; (b) transcribing isolated RNA to cDNA; (c) amplifying separate cDNA sequences encoding a heavy and a light variable region of the monoclonal antibody by a polymerase chain reaction; (d) cloning of separate amplification products encoding the heavy and the light variable regions, respectively, into vector constructs; (e) cloning of DNA encoding the heavy and the light variable regions, wherein the separate sequences are joined through a linker nucleotide sequence, into a vector construct; and (f) digesting the clones with appropriate restriction endonucleases. Preferably, the process of the invention utilizes a linker sequence that is the polynucleotide sequence described by SEQ ID NO:*7*.

Preferably, the isolated and purified polynucleotide of the claimed invention encodes a polypeptide comprising an amino acid residue sequence according to SEQ ID NO:*20, 21 or 22*. More preferably, the isolated and purified polynucleotide of the invention comprises a nucleotide sequence according to SEQ ID NO:*23, 24 or 25*.

In an alternative embodiment, the present invention provides an isolated and purified polynucleotide comprising a nucleotide base sequence that is identical or complimentary to a segment of at least 10 contiguous nucleotide bases of SEQ ID NO:*23, 24 or 25*, wherein the polynucleotide hybridizes to a polynucleotide that encodes a single chain variable region polypeptide that binds to a CD19 antigen. Preferably, the

polynucleotide of this embodiment of the invention encodes a polypeptide that has a molecular weight of approximately 28 kDa. More preferably still, the encoded polypeptide binds to a CD19 antigen with a K_a of at least 1 x 10^9 M^{-1}.

In another aspect, this embodiment of the invention provides an isolated and purified polynucleotide comprising a nucleotide base sequence that is identical or complimentary to a segment of 25 or 50 or 100 contiguous nucleotide bases of SEQ ID NO:*23, 24 or 25,* wherein the polynucleotide hybridizes to a polynucleotide that encodes a single chain variable region polypeptide that binds to a CD19 antigen.

In yet another embodiment, the present invention contemplates an isolated and purified single chain variable region polypeptide that binds to a CD19 antigen. Preferably, the polypeptide of this embodiment has a molecular weight of approximately 28 kDa. More preferably, the polypeptide binds to a CD19 antigen with a K_a of at least 1 x 10^9 M^{-1}. More preferably still, the polypeptide comprises an amino acid residue sequence according to SEQ ID NO:*20, 21 or 22.*

In another aspect of this embodiment, the isolated and purified polypeptide of the claimed invention is further modified by the site specific insertion of a cysteine residue at the C-terminus of the polypeptide. Also contemplated by the invention is a dimer of an isolated and purified single chain variable region polypeptide, wherein the dimer is prepared by linking a first polypeptide modified by the site specific insertion of a cysteine residue at the C-terminus of the polypeptide with a second polypeptide modified by the site specific insertion of a cysteine residue at the C-terminus of the polypeptide, the linking accomplished through a disulfide bond between a C-terminus cysteine residue on each polypeptide. In yet another embodiment, the present invention provides a process for preparing an isolated and purified single chain variable region polypeptide that binds to a CD19 antigen comprising the steps of (a) cloning a DNA sequence that encodes the polypeptide into an expression vector; (b) transforming *E. coli* cells with the expression vector; and (c) maintaining

the transformed cells under biological conditions sufficient for expression of the polypeptide. Preferably, the process of this embodiment uses *E. coli* cells from a BL21(DE3) strain.

In another aspect, this embodiment of the invention provides an isolated and purified single chain variable region polypeptide that binds to a CD19 antigen, wherein the polypeptide is preparable by a process comprising the steps of (a) cloning a DNA sequence that encodes the polypeptide into an expression vector; (b) transforming *E. coli* cells with the expression vector; and (c) maintaining the transformed cells under biological conditions sufficient for expression of the polypeptide.

Preferably, this aspect of the invention provides an isolated and purified single chain variable region polypeptide that binds to a CD19 antigen, wherein the polypeptide is prepared by a process comprising the steps of (a) cloning a DNA sequence that encodes the polypeptide into an expression vector; (b) transforming *E. coli* cells with the expression vector; and (c) maintaining the transformed cells under biological conditions sufficient for expression of the polypeptide.

In an alternative aspect, the present invention contemplates a polypeptide prepared as described immediately above, wherein the polypeptide is further modified by the site specific insertion of a cysteine residue at the C-terminus of the polypeptide. This aspect also provides a dimer of an isolated and purified single chain variable region polypeptide, wherein the dimer is prepared by linking a first polypeptide modified by the site specific insertion of a cysteine residue at the C-terminus of the polypeptide with a second polypeptide modified by the site specific insertion of a cysteine residue at the C-terminus of the polypeptide, the linking accomplished through a disulfide bond between a C-terminus cysteine residue on each polypeptide.

Also contemplated by the present invention is a polypeptide comprising an amino acid residue sequence of from five to sixty contiguous amino acid residues identical to any five to sixty contiguous amino acid residues of the polypeptide as defined by SEQ ID NO:*20, 21 or*

22, wherein the polypeptide retains an ability to bind to a CD19 antigen with a K_a of at least 1 x 10^9 M^{-1}.

In an alternative embodiment, the claimed invention provides an immunoconjugate for the treatment of cancer comprising a single chain variable region polypeptide that binds to a CD19 antigen, wherein the polypeptide is linked to at least one cytotoxic agent. Preferably, the cancer susceptible to treatment with the immunoconjugate of the invention is a B-cell leukemia. More preferably, the immunoconjugate of this embodiment of the invention comprises an amino acid residue sequence according to SEQ ID NO:*20, 21 or 22.* Alternatively, the cytotoxic agent of the immunoconjugate of the invention is selected from the group consisting of single chain, double chain, and multiple chain toxins. In another aspect, the cytotoxic agent of the immunoconjugate of the invention is selected from the group consisting of beta-emitting metallic radionuclides, alpha emitters, and gamma emitters.

In yet another embodiment, the present invention provides an immunoconjugate for the treatment of cancer comprising a polypeptide that is a dimer of an isolated and purified single chain variable region polypeptide, wherein the dimer is prepared by linking a first polypeptide modified by the site specific insertion of a cysteine residue at the C-terminus of the polypeptide with a second polypeptide modified by the site specific insertion of a cysteine residue at the C-terminus of the polypeptide, the linking accomplished through a disulfide bond between a C-terminus cysteine residue on each polypeptide. and wherein the polypeptide is linked to at least one cytotoxic agent. Preferably, this immunoconjugate of the invention is efficacious for the treatment of B-cell leukemia. More preferably, the cytotoxic agent of this immunoconjugate is selected from the group consisting of single chain, double chain, and multiple chain toxins. Alternatively, the cytotoxic agent is a radionuclide selected from the group consisting of beta-emitting metallic radionuclides, alpha emitters, and gamma emitters. In another aspect, the immunoconjugate comprises both a toxin and a radionuclide.

The present invention also contemplates a process for preparing an immunoconjugate comprising a single chain variable region polypeptide that binds to a CD19 antigen, wherein the process comprises the steps of (1) preparing the polypeptide according to a method comprising the steps of (a) cloning a DNA sequence that encodes the polypeptide into an expression vector; (b) transforming *E. coli* cells with the expression vector; and (c) maintaining the transformed cells under biological conditions sufficient for expression of the polypeptide; (2) providing a suitable toxin; and (3) conjugating the polypeptide to the toxin. As contemplated by the present invention, the process also further comprises the step of labelling the immunoconjugate with a radionuclide. Preferably, the toxin used in the process of the invention is selected from the group consisting of single chain, double chain, and multiple chain toxins. Likewise, the radionuclide used in the claimed process is selected from the group consisting of beta-emitting metallic radionuclides, alpha emitters, and gamma emitters. In another aspect of this embodiment, the polypeptide of the immunoconjugate comprises an amino acid residue sequence according to SEQ ID NO:*20, 21 or 22*.

Alternatively, the present invention provides an immunoconjugate for the treatment of cancer comprising a single chain variable region polypeptide that binds to a CD19 antigen, wherein the immunoconjugate is preparable by a process comprising the steps of (1) preparing the polypeptide according to a method comprising the steps of (a) cloning a DNA sequence that encodes the polypeptide into an expression vector; (b) transforming *E. coli* cells with the expression vector; and (c) maintaining the transformed cells under biological conditions sufficient for expression of the polypeptide; (2) providing a suitable toxin; and (3) conjugating the polypeptide to the toxin. Preferably, the immunoconjugate for the treatment of cancer comprising a single chain variable region polypeptide that binds to a CD19 antigen is prepared by a process comprising the steps of (1) preparing the polypeptide according to a method comprising the steps of (a) cloning a DNA sequence that encodes

the polypeptide into an expression vector; (b) transforming *E. coli* cells with the expression vector; and (c) maintaining the transformed cells under biological conditions sufficient for expression of the polypeptide; (2) providing a suitable toxin; and (3) conjugating the polypeptide to the toxin.

Also provided is the immunoconjugate described immediately above, wherein the polypeptide of the immunoconjugate is linked to at least one cytotoxic agent. Preferably, the cytotoxic agent is selected from the group consisting of single chain, double chain, and multiple chain toxins or, alternatively, the cytotoxic agent is a radionuclide selected from the group consisting of beta-emitting metallic radionuclides, alpha emitters, and gamma emitters, or the immunoconjugate comprises one of each type of cytotoxic agent. Such an immunoconjugate is contemplated to be efficacious in the treatment of B-cell leukemia.

The present invention further contemplates an additional embodiment of a method for the treatment of cancer comprising the steps of (a) selecting a patient evidencing symptoms of a B-cell cancer, wherein the cancer is selected from the group consisting of leukemia and B-cell lymphoma; (b) administering to the patient, in a biocompatible dosage form, a therapeutically effective amount of an immunoconjugate, prepared according to a process comprising the steps of (1) preparing a polypeptide according to a method comprising the steps of (a) cloning a DNA sequence that encodes the polypeptide into an expression vector; (b) transforming *E. coli* cells with the expression vector; and (c) maintaining the transformed cells under biological conditions sufficient for expression of the polypeptide; (2) providing a suitable toxin; (3) conjugating the polypeptide to the toxin; and (4) labelling the immunoconjugate with a radionuclide. Preferably, the radionuclide with which the immunoconjugate is labelled is ^{131}I.

In yet another embodiment, the present invention provides for a method for the treatment of cancer comprising the steps of (a) selecting a patient evidencing symptoms of a B-cell cancer, wherein the cancer is selected from the group consisting of leukemia and B-cell lymphoma; and

(b) administering to the patient, in a biocompatible dosage form, a therapeutically effective amount of an immunoconjugate comprising a polypeptide that is a dimer of an isolated and purified single chain variable region polypeptide, wherein the dimer is prepared by linking a first polypeptide modified by the site specific insertion of a cysteine residue at the C-terminus of the polypeptide with a second polypeptide modified by the site specific insertion of a cysteine residue at the C-terminus of the polypeptide, the linking accomplished through a disulfide bond between a C-terminus cysteine residue on each polypeptide. and wherein the polypeptide is linked to a toxin and labelled with a radionuclide.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. *Cloning strategy for development of anti CD19 scFv.*

The variable domain of the heavy chain and the linker which encodes $(G_4S)_3$ were ligated into Bluescript K5 plasmid at *Xho1* and *Sac1* sites. Variable domains of the light chain were inserted into *Sst1* and *BglII* sites following the linker. The pERT vector which was constructed by modifying pET3b was used as the expression vector for scFv. The nucleotides between *Ndcl* and *Xho1* sites of pERT encode four amino acids which are part of the FR1 of V_H but not included in the PCR products of V_H. The scFv encoding fragment was cloned into the pERT vector at *Xho1* and *Bgl II* sites. Positive clones were identified by restriction enzyme analysis and DNA sequencing.

Figure 2. *Comparison of the DNA sequence of the different variable regions from the heavy and light chains* (in two panels).

A: Heavy chain sequence. B: Light chain sequence. In the heavy chain CDR3, lower case letters are *n* nucleotide additions and they flank the germline encoded D_H gene sequences. Capitol letters indicate primers used in PCR.

Figure 3. *Amino acid sequence alignment of the variable heavy and light chain regions from the three different hybridomas: B43, 25C1 and BLY3* (in two panels).

Sequence differences are as indicated. The predicted protein sequences from the primers used for PCR are shown in bold type.

Figure 4. *Expression and Purification of scFv.*

Lane 1, Molecular weight markers (97, 66, 45, 31, 21 KD); Lane 2, Uninduced cells; Lane 3, Induced cells; Lane 4, Sonicated supernatant; Lane 5, Detergent-solubilized supernatant; Lane 6, Pellet; Lane 7, Pellet purified by Q sepharose.

Figure 5. *Specific binding of FVS191 and FVS192 to CD19+ HLA Class I+ Cells in FACS.*

The X axis represents binding of FITC labelled class I antibody, Y axis represents binding of phycoerythrin labelled CD19 antibody. Panel A, negative control; panel B, positive control; panel C, specific blocking with FVS191; panel D, specific blocking with FVS192.

Figure 6. *Scatchard analysis of binding of FVS191.*

Results are plotted with molecules/cell on horizontal axis and molecule L per cell mole on vertical axis. The derived K_a is 2 X 10^9.

DETAILED DESCRIPTION OF THE INVENTIO

There is a great need for the development of new therapeutic reagents for the treatment of a variety of diseases that are refractory to current therapies; one approach to developing these therapies has been through the use of monoclonal antibodies. The use of monoclonal antibodies in leukemia is particularly attractive because specific subsets of

cells may be potentially specifically targeted. Several approaches have been tried using monoclonal antibodies for therapeutic use and often rely on the ability to chemically conjugate the antibodies to toxins (Ghetie *et al.*, 1988; Uckun *et al.*, 1986; Myers *et al.*, 1991; Jansen *et al.*, 1992). However, there are several disadvantages to use of intact antibodies particularly because of the large size of the molecules and the resultant relative inability to penetrate tissues (Pastan *et al.*, 1991; Yokota *et al.*, 1992).

Single chain fragments have been developed to overcome the problems associated with intact antibodies. scFvs contain only the variable regions from the heavy and light chains and have a molecular mass of approximately 28 kDa compared to that of the intact antibody of 150 kDa. However, many scFvs expressed in bacteria are insoluble, difficult to refold, and their ability to retain binding to the antigen of interest is highly variable. Because the effects of primary amino acid sequence on protein folding are not well understood, there is no known *a priori* method for determining the ability of a particular antibody to function when produced as an scFv. Accordingly, scFvs developed from three hybridomas that produce antibodies that bind to the CD19 antigen of B cells have been cloned and expressed.

Polynucleotides and Methods of the Invention.

In a first aspect, the present invention provides an isolated and purified polynucleotide encoding a single chain variable region polypeptide that binds to a CD19 antigen. Preferably, the isolated and purified polynucleotide of the invention encodes a polypeptide that has a molecular weight of approximately 28 kDa. More preferably, the polynucleotide the invention encodes a polypeptide that binds to a CD19 antigen with a K_a of at least 1×10^9 M^{-1}. As used herein, the term "polynucleotide" means a sequence of nucleotides connected by phosphodiester linkages.

Also provided by the invention is a process for preparing an isolated and purified polynucleotide encoding a single chain variable region polypeptide that binds to a CD19 antigen comprising the steps of (a)

isolating RNA from hybridomas producing monoclonal antibodies to CD19 antigen; (b) transcribing isolated RNA to cDNA; (c) amplifying separate cDNA sequences encoding a heavy and a light variable region of the monoclonal antibody by a polymerase chain reaction; (d) cloning of separate amplification products encoding the heavy and the light variable regions, respectively, into vector constructs; (e) cloning of DNA encoding the heavy and the light variable regions, wherein the separate sequences are joined through a linker nucleotide sequence, into a vector construct; and (f) digesting the clones with appropriate restriction endonucleases. Preferably, the process of the invention utilizes a linker sequence that is the polynucleotide sequence described by SEQ ID NO:7.

The present invention also contemplates an isolated and purified polynucleotide preparable by a process comprising the steps of (a) isolating RNA from hybridomas producing monoclonal antibodies to CD19 antigen; (b) transcribing isolated RNA to cDNA; (c) amplifying separate cDNA sequences encoding a heavy and a light variable region of the monoclonal antibody by a polymerase chain reaction; (d) cloning of separate amplification products encoding the heavy and the light variable regions, respectively, into vector constructs; (e) cloning of DNA encoding the heavy and the light variable regions, wherein the separate sequences are joined through a linker nucleotide sequence, into a vector construct; and (f) digesting the clones with appropriate restriction endonucleases.

Alternatively, the present invention provides an isolated and purified polynucleotide prepared by a process comprising the steps of (a) isolating RNA from hybridomas producing monoclonal antibodies to CD19 antigen; (b) transcribing isolated RNA to cDNA; (c) amplifying separate cDNA sequences encoding a heavy and a light variable region of the monoclonal antibody by a polymerase chain reaction; (d) cloning of separate amplification products encoding the heavy and the light variable regions, respectively, into vector constructs; (e) cloning of DNA encoding the heavy and the light variable regions, wherein the separate sequences are joined through a linker nucleotide sequence, into a vector construct;

and (f) digesting the clones with appropriate restriction endonucleases. Preferably, the process of the invention utilizes a linker sequence that is the polynucleotide sequence described by SEQ ID NO:*7*. More preferably, the isolated and purified polynucleotide of the claimed invention encodes a polypeptide comprising an amino acid residue sequence according to SEQ ID NO:*20, 21 or 22*. More preferably still, the isolated and purified polynucleotide of the invention comprises a nucleotide sequence according to SEQ ID NO:*23, 24 or 25*.

In an alternative embodiment, the present invention provides an isolated and purified polynucleotide comprising a nucleotide base sequence that is identical or complimentary to a segment of at least 10 contiguous nucleotide bases of SEQ ID NO:*23, 24 or 25*, wherein the polynucleotide hybridizes to a polynucleotide that encodes a single chain variable region polypeptide that binds to a CD19 antigen. Preferably, the polynucleotide of this embodiment of the invention encodes a polypeptide that has a molecular weight of approximately 28 kDa. More preferably still, the encoded polypeptide binds to a CD19 antigen with a K_a of at least 1×10^9 M^{-1}.

In another aspect, this embodiment of the invention provides an isolated and purified polynucleotide comprising a nucleotide base sequence that is identical or complimentary to a segment of 25 or 50 or 100 contiguous nucleotide bases of SEQ ID NO:*23, 24 or 25*, wherein the polynucleotide hybridizes to a polynucleotide that encodes a single chain variable region polypeptide that binds to a CD19 antigen.

Polypeptides and Methods of the Invention.

The scFv polypeptides developed from three hybridomas were expressed at high levels in bacteria. No instability of the protein, as determined by examination of Coomassie stained SDS-PAGE gels, was noted over the period of induction (3 hrs.) and all clones produced approximately the same quantities of protein. However, the ability of the scFv from each of these clones to bind to the target antigen varied greatly. Although the BLy3 and B43 hybridomas produced heavy chain and light

chain variable proteins that were from the same family, only the protein produced from the B43 clone (FVS191) was able to show any ability to bind to the CD19 protein. This indicates the importance of the total sequence in the refolding of the native protein structure but indicates that development of scFv with proper folding and high binding affinity remains empiric. Like FVS191, the scFv clone from 25C1 (FVS192) also produced a protein capable of recognizing the antigen. However, the specific affinity of FVS192 for the CD19 antigen was low and could not be quantified in Scatchard analyses.

In yet another embodiment, the present invention contemplates an isolated and purified single chain variable region polypeptide that binds to a CD19 antigen. Preferably, the polypeptide of this embodiment has a molecular weight of approximately 28 kDa. More preferably, the polypeptide binds to a CD19 antigen with a K_a of at least 1×10^9 M^{-1}. More preferably still, the polypeptide comprises an amino acid residue sequence according to SEQ ID NO:*20, 21 or 22*.

In another aspect of this embodiment, the isolated and purified polypeptide of the claimed invention is further modified by the site specific insertion of a cysteine residue at the C-terminus of the polypeptide. Also contemplated by the invention is a dimer of an isolated and purified single chain variable region polypeptide, wherein the dimer is prepared by linking a first polypeptide modified by the site specific insertion of a cysteine residue at the C-terminus of the polypeptide with a second polypeptide modified by the site specific insertion of a cysteine residue at the C-terminus of the polypeptide, the linking accomplished through a disulfide bond between a C-terminus cysteine residue on each polypeptide. As used herein the term "polypeptide" means a polymer of amino acids connected by amide linkages, wherein the number of amino acid residues can range from about 5 to about one million. Preferably, a polypeptide has from about 10 to about 1000 amino acid residues and, even more preferably from about 20 to about 500 amino residues. Thus, as used herein, a polypeptide includes what is often referred to in the art as an oligopeptide

(5-10 amino acid residues), a polypeptide (11-100 amino acid residues) and a protein (>100 amino acid residues). A polypeptide encoded by an encoding region can undergo post-translational modification to form conjugates with carbohydrates, lipids, nucleic acids and the like to form glycopolypeptides (e.g., glycoproteins), lipopolypeptides (e.g. lipoproteins) and other like conjugates.

Polypeptides are disclosed herein as amino acid residue sequences. Those sequences are written left to right in the direction from the amino to the carboxy terminus. In accordance with standard nomenclature, amino acid residue sequences are denominated by either a single letter or a three letter code as indicated in Table 1 below.

TABLE 1

Amino Acid Residue	3-Letter Code	1-Letter Code
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic Acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic Acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Seriune	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

Modifications and changes may be made in the structure of a polypeptide of the present invention and still obtain a molecule having like characteristics. For example, certain amino acids can be substituted for other amino acids in a sequence without appreciable loss of activity. Because it is the interactive capacity and nature of a polypeptide that defines that polypeptide's biological functional activity, certain amino acid

sequence substitutions can be made in a polypeptide sequence (or, of course, its underlying DNA coding sequence) and nevertheless obtain a polypeptide with like properties.

In making such changes, the hydropathic index of amino acids can be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a polypeptide is generally understood in the art (Doolittle, *et al.* 1982). It is known that certain amino acids can be substituted for other amino acids having a similar hydropathic index or score and still result in a polypeptide with similar biological activity. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics.

Those indices are given in Table 2, below.

TABLE 2

Amino Acid	Index	Amino Acid	Index
isoleucine	(+4.5)	tryptophan	(-0.9)
valine	(+4.2)	tyrosine	(-1.3)
leucine	(+3.8)	proline	(-1.6)
phenylalanine	(+2.8)	histidine	(-3.2)
cysteine	(+2.5)	glutamate	(-3.5)
methionine	(+1.9)	glutamine	(-3.5)
alanine	(+1.8)	aspartate	(-3.5)
glycine	(-0.4)	asparagine	(-3.5)
threonine	(-0.7)	lysine	(-3.9)
serine	(-0.8)	arginine	(-4.5)

It is believed that the relative hydropathic character of the amino acid determines the secondary structure of the resultant polypeptide, which in turn defines the interaction of the polypeptide with other molecules, for example, enzymes, substrates, receptors, antibodies, antigens, and the like. It is known in the art that an amino acid may be substituted by another amino acid having a similar hydropathic index and still obtain a biologically functionally equivalent polypeptide. In such

changes, the substitution of amino acids whose hydropathic indices are within ± 2 is preferred, those which are within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred.

Substitution of like amino acids can also be made on the basis of hydrophilicity, particularly where the biologically functionally equivalent peptide or polypeptide thereby created is intended for use in immunological embodiments. U.S. Patent 4,554,101, incorporated herein by reference, states that the greatest local average hydrophilicity of a polypeptide, as governed by the hydrophilicity of its adjacent amino acids, correlate with its immunogenicity and antigenicity, *i.e.* with a biological property of the polypeptide.

As detailed in U.S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 ± 1); glutamate (+3.0 ± 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); proline (-0.5 ± 1); threonine (-0.4); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent, polypeptide. In such changes, the substitution of amino acids whose hydrophilicity values are within ±2 is preferred, those which are within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred.

As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions which take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine. The present invention thus contemplates functional equivalents of the

claimed polypeptides.

In yet another embodiment, the present invention provides a process for preparing an isolated and purified single chain variable region polypeptide that binds to a CD19 antigen comprising the steps of (a) cloning a DNA sequence that encodes the polypeptide into an expression vector; (b) transforming *E. coli* cells with the expression vector; and (c) maintaining the transformed cells under biological conditions sufficient for expression of the polypeptide. Preferably, the process of this embodiment uses *E. coli* cells from a BL21(DE3) strain.

In another aspect, this embodiment of the invention provides an isolated and purified single chain variable region polypeptide that binds to a CD19 antigen, wherein the polypeptide is preparable by a process comprising the steps of (a) cloning a DNA sequence that encodes the polypeptide into an expression vector; (b) transforming *E. coli* cells with the expression vector; and (c) maintaining the transformed cells under biological conditions sufficient for expression of the polypeptide.

Preferably, this aspect of the invention provides an isolated and purified single chain variable region polypeptide that binds to a CD19 antigen, wherein the polypeptide is prepared by a process comprising the steps of (a) cloning a DNA sequence that encodes the polypeptide into an expression vector; (b) transforming *E. coli* cells with the expression vector; and (c) maintaining the transformed cells under biological conditions sufficient for expression of the polypeptide.

In an alternative aspect, the present invention contemplates a polypeptide prepared as described immediately above, wherein the polypeptide is further modified by the site specific insertion of a cysteine residue at the C-terminus of the polypeptide. This aspect also provides a dimer of an isolated and purified single chain variable region polypeptide, wherein the dimer is prepared by linking a first polypeptide modified by the site specific insertion of a cysteine residue at the C-terminus of the polypeptide with a second polypeptide modified by the site specific insertion of a cysteine residue at the C-terminus of the polypeptide, the

linking accomplished through a disulfide bond between a C-terminus cysteine residue on each polypeptide.

Also contemplated by the present invention is a polypeptide comprising an amino acid residue sequence of from five to sixty contiguous amino acid residues identical to any five to sixty contiguous amino acid residues of the polypeptide as defined by SEQ ID NO:*20, 21 or 22*, wherein the polypeptide retains an ability to bind to a CD19 antigen with a K_a of at least 1×10^9 M^{-1}.

Immunoconjugates and Methods of the Invention.

In an alternative embodiment, the claimed invention provides an immunoconjugate for the treatment of cancer comprising a single chain variable region polypeptide that binds to a CD19 antigen, wherein the polypeptide is linked to at least one cytotoxic agent. Preferably, the cancer susceptible to treatment with the immunoconjugate of the invention is a B-cell leukemia. More preferably, the immunoconjugate of this embodiment of the invention comprises an amino acid residue sequence according to SEQ ID NO:*20, 21 or 22*. Alternatively, the cytotoxic agent of the immunoconjugate of the invention is selected from the group consisting of single chain, double chain, and multiple chain toxins. In another aspect, the cytotoxic agent of the immunoconjugate of the invention is selected from the group consisting of beta-emitting metallic radionuclides, alpha emitters, and gamma emitters.

Toxins

A structural similarity in plant and bacterial toxins inhibits protein synthesis: they are usually heterodimers made of a polypeptide chain (B chain) that binds the toxin to target cells and a second chain (A chain) that displays enzymatic activity (Olsnes *et al.*, 1982). The two chains are linked by a disulfide bond. Diphtheria toxin is a slight exception in that a single proteolytic cleavage is required to generate an A and a B chain (Collier *et al.*, 1971) that are also disulfide bonded. In addition, it is provocative that the subunits of all the plant toxins have approximately the same apparent molecular weight (Olsnes *et al.*, 1982; Olsnes *et al.*, 1974), about 30,000, that

the A chains attack the 60S ribosomal subunit (Olsnes *et al.*, 1982; Olsnes *et al.*, 1974; Olsnes *et al.*, 1984) and the B chains bind to galactose (Olsnes *et al.*, 1982; Olsnes *et al.*, 1974; Olsnes *et al.*, 1984). Moreover, the A and B chains of abrin and ricin, two toxins derived from phylogenetically distant plants, can be interchanged to produce hybrid molecules of relatively high toxicity (Olsnes *et al.*, 1982; Olsnes *et al.*, 1984). These observations suggest significant conservation in function and structure. Whether the structural conservation is at the three-dimensional level only or reflects primary amino acid sequence homologies remains to be determined. There is also a variety of plant toxins composed of A chains only, *e.g.*, gelonin (Stirpe *et al.*, 1980) and pokeweed antiviral protein (PAP) (Olsnes *et al.*, 1982; Barbieri *et al.*, 1982). These A chains function in the same way as the A chains of intact toxins.

In yet another embodiment, the present invention provides an immunoconjugate for the treatment of cancer comprising a polypeptide that is a dimer of an isolated and purified single chain variable region polypeptide, wherein the dimer is prepared by linking a first polypeptide modified by the site specific insertion of a cysteine residue at the C-terminus of the polypeptide with a second polypeptide modified by the site specific insertion of a cysteine residue at the C-terminus of the polypeptide, the linking accomplished through a disulfide bond between a C-terminus cysteine residue on each polypeptide. and wherein the polypeptide is linked to at least one cytotoxic agent. Preferably, this immunoconjugate of the invention is efficacious for the treatment of B-cell leukemia. More preferably, the cytotoxic agent of this immunoconjugate is selected from the group consisting of single chain, double chain, and multiple chain toxins. Alternatively, the cytotoxic agent is a radionuclide selected from the group consisting of beta-emitting metallic radionuclides, alpha emitters, and gamma emitters. In another aspect, the immunoconjugate comprises both a toxin and a radionuclide.

The present invention also contemplates a process for preparing an immunoconjugate comprising a single chain variable region polypeptide

that binds to a CD19 antigen, wherein the process comprises the steps of (1) preparing the polypeptide according to a method comprising the steps of (a) cloning a DNA sequence that encodes the polypeptide into an expression vector; (b) transforming *E. coli* cells with the expression vector; and (c) maintaining the transformed cells under biological conditions sufficient for expression of the polypeptide; (2) providing a suitable toxin; and (3) conjugating the polypeptide to the toxin. As contemplated by the present invention, the process also further comprises the step of labelling the immunoconjugate with a radionuclide. Preferably, the toxin used in the process of the invention is selected from the group consisting of single chain, double chain, and multiple chain toxins. Likewise, the radionuclide used in the claimed process is selected from the group consisting of beta-emitting metallic radionuclides, alpha emitters, and gamma emitters. In another aspect of this embodiment, the polypeptide of the immunoconjugate comprises an amino acid residue sequence according to SEQ ID NO:*20, 21 or 22*.

The toxins which are usable in the practice of the claimed invention encompass all toxins used in the production of immunotoxins. Generally, the toxins include heterodimers made of a polypeptide chain (B chain) that binds the toxin to target cells via a sugar on the surface and a second chain (A chain) that displays enzymatic activity. The two chains are typically linked by a disulfide bond. Examples of two chain toxins are ricin, abrin, modeccin, diphtheria toxin and viscumin. However, single chain toxins, *i.e.* toxins composed of A chains only, *e.g.*, gelonin, pseudomonas aeruginosa Exotoxin A, and amanitin may also be utilized. Other single chain toxins are hemitoxins which are also usable in this invention. They include pokeweed antiviral protein (PAP), saporin and memordin. Other useful single chain toxins include the A-chain fragments of the two chain toxins. A chain toxins with multiple B chains such as Shigella toxin are also usable in the invention.

As used herein, 2-chain toxins refers to toxins formed from two chains, and single chain toxins refers to both toxin obtained by cleaving 2-

chain toxins as well as toxins having only one chain.

A preferred toxin is ricin, a toxin lectin extracted from the seeds of Ricinus communis, which contains an enzymatic and protein synthesis inhibiting A chain and a B chain which contains galactose binding site(s). Ricin is extremely toxic and it has been calculated that a single molecule of ricin in the cytosol will kill a cell. Ricin may be obtained and purified by the procedures described in U.S. Pat. No. 4,340,535, the disclosure of which is incorporated herein by reference.

Alternatively, the present invention provides an immunoconjugate for the treatment of cancer comprising a single chain variable region polypeptide that binds to a CD19 antigen, wherein the immunoconjugate is preparable by a process comprising the steps of (1) preparing the polypeptide according to a method comprising the steps of (a) cloning a DNA sequence that encodes the polypeptide into an expression vector; (b) transforming *E. coli* cells with the expression vector; and (c) maintaining the transformed cells under biological conditions sufficient for expression of the polypeptide; (2) providing a suitable toxin; and (3) conjugating the polypeptide to the toxin. Preferably, the immunoconjugate for the treatment of cancer comprising a single chain variable region polypeptide that binds to a CD19 antigen is prepared by a process comprising the steps of (1) preparing the polypeptide according to a method comprising the steps of (a) cloning a DNA sequence that encodes the polypeptide into an expression vector; (b) transforming *E. coli* cells with the expression vector; and (c) maintaining the transformed cells under biological conditions sufficient for expression of the polypeptide; (2) providing a suitable toxin; and (3) conjugating the polypeptide to the toxin. One general method of preparing immunotoxins is to use a thiol-containing heterobifunctional crosslinker, *e.g.*, SPDP, which attacks primary amino groups on the antibody and by disulfide exchange can attach either the SH-containing A chain or the SPDP-derivatized holotoxin to the antibody (Cumber *et al.*, 1984; Carlsson *et al.*, 1978). If the disulfide exchange is carried out at neutral pH a relatively stable disulfide bond is

formed and the conjugate remains intact when incubated with fresh mouse serum *in vitro*.

The nature of the linkage between the A chain and the antibody or fragment is of critical important in determining toxicity. If the bond cannot be broken readily in an endosome/phagolysosome (Jansen *et al.*, 1982; Ramakrishnan *et al.*, 1984), *e.g.*, a stable thioether bond, then toxicity is virtually abolished (Jansen *et al.*, 1982). In contrast, if the bond is highly unstable, then the conjugate may dissociate either before it reaches the target cell or, perhaps, prematurely within the target cell. In the latter case, the A chain may be degraded before translocation can occur.

Also provided is the immunoconjugate described above, wherein the polypeptide of the immunoconjugate is linked to at least one cytotoxic agent. Preferably, the cytotoxic agent is selected from the group consisting of single chain, double chain, and multiple chain toxins or, alternatively, the cytotoxic agent is a radionuclide selected from the group consisting of beta-emitting metallic radionuclides, alpha emitters, and gamma emitters, or the immunoconjugate comprises one of each type of cytotoxic agent. Such an immunoconjugate is contemplated to be efficacious in the treatment of B-cell leukemia.

Among the radionuclides used, gamma-emitters, positron-emitters, and X-ray emitters are suitable for localization and/or therapy, while beta emitters and alpha emitters may also be used for therapy. Suitable radionuclides for forming the immunoconjugate of the invention include ^{123}I, ^{125}I, ^{130}I, ^{131}I, ^{133}I, ^{135}I, ^{47}Sc, ^{72}As, ^{72}Se, ^{90}Y, ^{88}Y, ^{97}Ru, ^{100}Pd, ^{101m}Rh, ^{119}Sb, ^{128}Ba, ^{197}Hg, ^{211}At, ^{212}Bi, ^{212}Pb, ^{109}Pd, ^{111}In, ^{67}Ga, ^{68}Ga, ^{67}Cu, ^{75}Br, ^{77}Br, ^{99m}Tc, ^{11}C, ^{13}N, ^{15}O and ^{18}F.

Methods for the Treatment of Cancer.

The present invention further contemplates an additional embodiment of a method for the treatment of cancer comprising the steps of (a) selecting a patient evidencing symptoms of a B-cell cancer, wherein the cancer is selected from the group consisting of leukemia and B-cell lymphoma; (b) administering to the patient, in a biocompatible dosage

form, a therapeutically effective amount of an immunoconjugate, prepared according to a process comprising the steps of (1) preparing a polypeptide according to a method comprising the steps of (a) cloning a DNA sequence that encodes the polypeptide into an expression vector; (b) transforming *E. coli* cells with the expression vector; and (c) maintaining the transformed cells under biological conditions sufficient for expression of the polypeptide; (2) providing a suitable toxin; (3) conjugating the polypeptide to the toxin; and (4) labelling the immunoconjugate with a radionuclide. Preferably, the radionuclide with which the immunoconjugate is labelled is ^{131}I.

In yet another embodiment, the present invention provides for a method for the treatment of cancer comprising the steps of (a) selecting a patient evidencing symptoms of a B-cell cancer, wherein the cancer is selected from the group consisting of leukemia and B-cell lymphoma; and (b) administering to the patient, in a biocompatible dosage form, a therapeutically effective amount of an immunoconjugate comprising a polypeptide that is a dimer of an isolated and purified single chain variable region polypeptide, wherein the dimer is prepared by linking a first polypeptide modified by the site specific insertion of a cysteine residue at the C-terminus of the polypeptide with a second polypeptide modified by the site specific insertion of a cysteine residue at the C-terminus of the polypeptide, the linking accomplished through a disulfide bond between a C-terminus cysteine residue on each polypeptide, and wherein the polypeptide is linked to a toxin and labelled with a radionuclide.

EXAMPLES

Example 1: Cloning and Expression of the scFv

A. *Cloning of the variable regions* (V_H and V_L)

Cells: The three anti-CD19 hybridomas used in these studies have been previously described: B43, produced by F. Uckun (Uckun *et al.*, 1986), SJ25C1, produced by S. Pieper, and BLY3, produced by S. Poppema (Knapp *et al.*, 1989b). All were maintained in RPMI 1640 supplemented with 10% fetal calf serum.

RNA was isolated by the method of Chomczynski and Sacchi (Chomczynski, 1987) and either used directly for RT-PCR or further purified by oligo dT column chromatograph. By way of example, and without limitation, the following protocol describes isolation of RNA from 100 mg of rat mammary tissue according to the method referenced above.

Immediately after removal from the animal, the tissue was minced on ice and homogenized (at room temperature) with 1 ml of solution D in a glass-Teflon homogenizer and subsequently transferred to a 4-ml polypropylene tube. Sequentially, 0.1 ml of 2M sodium acetate, pH 4, 1 ml of phenol (water saturated), and 0.2 ml of chloroform-isoamyl alcohol mixture (49:1) were added to the homogenate, with thorough mixing by inversion after the addition of each reagent. The final suspension was shaken vigorously for 10 s and cooled on ice for 15 min. Samples were centrifuged at 10,000g for 20 min. at 4°C. After centrifugation, RNA was present in the aqueous phase whereas DNA and proteins were present in the interphase and phenol phase. The aqueous phase was transferred to a fresh tube, mixed with 1 ml of isopropanol, and then placed at -20°C for at least 1 h to precipitate RNA. Sedimentation at 10,000g for 20 min. was again performed and the resulting RNA pellet was dissolved in 0.3 ml of solution D, transferred into a 1.5-ml Eppendorf tube, and precipitated with 1 vol of isopropanol at -20°C for 1 h. After centrifugation in an Eppendorf centrifuge for 10 min. at 4°C the RNA pellet was resuspended in 75% ethanol, sedimented, vacuum dried (15 min.), and dissolved in 50 µl 0.5% SDS at 65°C for 10 min. At this point the RNA preparation could be used for poly(A)+ selection by oligo (dT) chromatography, Northern blot analysis, and dot blot hybridization. Isopropanol precipitation can be replaced by precipitation with a double volume of ethanol.

Reverse transcription of the isolated RNA was performed according to the recommendations of the manufacturer (Life Technologies) using random hexamers and was performed in a 50 microliter reaction volume with 1-2 micrograms of polyadenylated RNA or 5-10 micrograms of total

RNA. Approximately 10 microliters of the reverse transcribed material was used for the polymerase chain reaction using one pair of the several different primers listed in Table 1. The primers Z221 and Z222 anneal to the constant regions of heavy and light chains, respectively, and were only used for isolating clones for verification of sequence but not for the production of variable regions that were subsequently used in the creation of the scFv. The cycle parameters were 1 cycle of 94°C for 5' before the addition of the *TaqI* polymerase then 30 cycles of 94° C 1' 30", 54°C 1' 30", 72°C 1', followed by 1 cycle of 94°C 1'30", 54°C 2'30", 72°C 10'. The PCR products were cloned either after treatment with Klenow into SmaI digested pBluescript or directly using the pCRI vector (Invitrogen) which has compatible T overhangs. Clones were identified based on the size of inserts (approximately 350bp for the V_L gene and 450bp for the V_H gene) and were confirmed by sequencing using standard dideoxynucleotide chain termination techniques (Sequenase, US Biochemicals). At least three different clones from three different PCR reactions were sequenced for each variable region to confirm the absence of any mutations induced by *Taq* polymerase before clones were used for the creation of scFV.

The DNA and the predicted amino acid sequences of the clones of the variable regions from the three hybridomas are shown in Fig. 2 and Fig. 3. As discussed in Materials and Methods, at least three clones from three independent PCR reactions were sequenced to ensure that no Taq-introduced mutations were present within the clones that were used for the scFv development. All heavy chain variable regions from the three hybridomas were from the J558 family which includes approximately 50% of all mouse heavy chain variable region genes (Brodeur *et al.*, 1984). Although clone 25CI uses J_H2, clones of B43 and BLy3 use J_H4. As expected, the B43 and Bly3 clones differed most within the CDR3 region due to N region differences.

Sequencing of the light chain variable regions showed that V_K21 was used in both B43 and Bly3 but V_K19 was used in 25C1. The J_K regions used were J_K1 for B43 and Bly3 and J_K2 for 25C1 (Sakano *et al.*, 1979). As

anticipated, the greatest region of variability was present in the CDR3 region due to differential splicing and N region additions. After clones without any apparent PCR-introduced mutations had been identified by sequence analysis, scFvs were constructed.

B. *Cloning of the scFv using V_H and V_L.*

The linker used in these studies was $(Gly_4Ser)_3$ as previously described (Huston *et al.*, 1988). The scFvs were created by ligation of the linker region oligonucleotides (Table 3) using the strategy outlined in FIG. 1. Heavy chain variable region was mixed simultaneously with linker and Bluescript to obtain the V_H-linker construct shown in FIG. 1. Clones that contained the heavy chain variable region were digested with *Xho*I and *BstE*II. Success of the procedure was confirmed by sequencing. Clones that contained the heavy chain variable region and the linker were then digested with *SstI* and *BglII* and ligated to gel purified light chain variable region that was digested with the same enzymes. Clones were identified by the appearance of appropriately sized restriction endonuclease fragments and finally by nucleotide sequence analysis. scFvs were then digested with *XhoI* and *BglII* and gel purified before ligation into the pERT expression vector.

TABLE 3. OLIGONUCLEOTIDES USED FOR scF_V CONSTRUCT

Primer Name	Oligonucleotide Sequence
5′ VH: Z462	AGGTCCAGCTGCTCGAGTCTGG I *Xho*1
3′ VH: B1867	TGAGGAGACGGTGACCGTGTCCCTTGGCCCCAG I *BstE*II
3′ VH: Z221	AGGCTTACTAGTACAATCCCTGGGCACAAT
5′ VK: Z407	CGCGGATCCAGTTCCGAGCTCGTGCTCACCCAGTCTCCA I *Sst*1
3′ VK: B1865	GAAGATCTACGTTTTATTTCCAGCTTGGTCCC I *Bgl*1
3′ VK: Z222	GCGCCGTCTAGAATTAACACTCATTCCTGTTGAA
Linker for V_H and V_L	GGAG GCGGTGG CTCGGGC GGTGGCG GCTCGGG TGGCGGC GGAT CC

The primers Z221, 222, 407 and 462 are based on sequemces from Huse et al. (1989).
The primers B1867 adn 1865 are based on primer sequences from Orlandi *et al.* (1989).
The * denotes primers that were used for the generation of clones used only for sequencing. The oligonucleotides used for the liner are based on the thses developed by Huston *et al.*

C. *Expression of scFvs.*

The vector used to express the scFv in these studies was developed using the pET3b plasmid established by Studier *et al.* (Studier *et al.*, 1990). This plasmid vector was developed for cloning and expressing target

DNAs under control of a T7 promoter and designated pET vectors (plasmid for expression by T7 RNA polymerase) (Rosenberg *et al.*, 1987). These vectors contain a T7 promoter inserted into the BamHI site of the multi-copy plasmid pBR322 in the orientation that transcription is directed counterclockwise, opposite to that from the TET promoter. In the absence of T7 RNA polymerase, transcription of target DNAs by *E. coli* RNA polymerase is low enough that very toxic genes can be cloned in these vectors. However, some expression can be detected, so it is possible that an occasional gene may be too toxic to be cloned in them.

Most of the pET vectors described confer resistance to ampicillin. In such vectors, the *bla* gene is oriented so that it will be expressed from the T7 promoter along with the target gene. However, in the pET-9 series of vectors, the *bla* gene has been replaced by *kan* gene in the opposite orientation. In these vectors, the only coding sequence transcribed from the T7 promoter is that of the target gene.

The T7 promoter in the pET vectors is derived from the ø10 promoter, one of six strong promoters in T7 DNA that have the identical nucleotide sequence from positions -17 to +6, where +1 is the position of the first nucleotide of the RNA transcribed from the promoter. The ø10 promoter fragments carried by the vectors all begin at bp -23 and continue to bp +2, +3, +26, and +96 or beyond. Some of the vectors also contain a transcription termination signal or an RNase III cleavage site downstream of the cloning site for the target DNA.

pET3b was modified to allow for the cloning and expression of the constructs of the present invention by ligating an oligonucleotide that coded for the first four amino acids (LESG) that are commonly found at the amino terminus of the heavy chain variable region to the vector that was digested with *NdeI* and *EcoRI*. This oligonucleotide also contained sequences for recognition sites for *XhoI, BglII, BamHI,* and *EcoRI* allowing for the cloning of the scFv into the vector at the *XhoI* and *BglII* sites with the possibility of cloning other potentially therapeutic genes in the future (Table 3).

Expression of protein was accomplished by introducing the scFv clones into either BL21(DE3) or BL21(DE3) pLysS *E.coli* cells. No difference in the amount of recombinant protein expressed by these host strains was observed. Induction of protein synthesis was performed with 1.0 mM IPTG for three hours prior to harvesting of cells. Pellets were boiled in SDS-Page loading buffer and subjected to electrophoresis in denaturing polyacrylamide gels (Laemmli, 1970). Bacteria could be successfully induced at an O.D. 600 nm. of 0.6-1.0 if grown in a standard Erlenmeyer flask or at an O.D. 600 nm. of 2.5-3.0 if grown in a Fernbach (baffled) flask. Although the amount of protein per cell did not appear to change between cells grown in either flask as determined by SDS-PAGE and Coomassie staining (data not shown) the total amount of protein was greater from cells grown in the baffled flasks due to their greater mass.

Constructs were used to direct the synthesis of protein in *E. coli* as described above. After induction the protein was subjected to SDS-PAGE and detected by staining with Coomassie brilliant blue (Fig. 4). The results show that a protein of the expected molecular weight (27.5kDa) was specifically induced by the addition of IPTG to the culture medium. This protein was not present in either control cells or cells that were not treated with IPTG.

Example 2: Isolation of Protein.

The isolation procedure for the scFvs followed that of previously published methods (Langley *et al.*, 1987). As described below, all of the protein produced was found within the insoluble cytoplasmic fraction presumably in inclusion bodies. Briefly, cells were harvested by centrifugation and washed in water before being resuspended in up to 1/5 of the original culture volume of 10 mM Tris-HCl pH 7.4, 50 mM NaCl. If the original culture volume was large (greater than 100mls) this solution was frozen at -20° C to ensure full lysis of the cells. The mixture was sonicated and centrifuged at 30,000x g for 30 minutes. The supernatant was discarded and the pellet was resuspended by sonication in 1/20 of the original culture volume in 10 mM Tris-HCl pH 8.0 and 5 mM EDTA.

After resuspension was complete the mixture was digested with 0.2% lysozyme (Sigma) for a minimum of 1hour. Finally, 1/3 volume of 10% sodium deoxycholate was added and the mixture was incubated at room temperature for 1 hour before centrifugation at 30,000 x g for 30 minutes. The pellet was washed three times in water by resuspending the pellet by sonication and centrifugation as described above. Pellets were either stored at -20° C or dissolved in 0.1M Tris-HCl ph 8.0, 6M guanidine HCl, 0.3M DTE, 2mM EDTA at room temperature for a minimum of 2hours. Refolding of the denatured scFv was performed according to the method of Buchner *et. al.* (15).

The protein concentration was measured using the Bradford assay and the solution was then rapidly diluted at least 100 fold to a concentration of 30 ug/ml protein in 0.1M Tris-HCl pH8.0, 0.5M L-arginine, 8mM GSSG and 2mM EDTA. After a minimum of 12 hours. at 10° C the refolded protein was concentrated using an Amicon spiral concentrator and spin concentrator before being chromatographed on Q Sepharose and finally Superose 75. As judged by the presence of a single peak on Superose chromatography and Coomassie stain of SDS-Page gels, protein was pure. If the concentration was too low for use in experiments the protein was concentrated by Amicon spin concentrators. Concentration of the protein was determined using the Bradford assay (BioRad) with bovine serum albumin as a standard.

To determine if the protein was present in an insoluble or soluble fraction, cells were disrupted by sonication and the supernatant and insoluble material separated by centrifugation. Analysis of the two fractions indicated that the bulk, if not all of the protein was present in the insoluble pellet. Due to the insolubility of the protein and its probable location within inclusion bodies, we performed isolations based on previously published methods for the purification of proteins from these vesicles. Refolded and purified protein was then used for FACS and Scatchard analysis.

Example 3: Analysis of the scFvs

I. FACS Analysis.

FACS analysis was performed on either RS4:11 (Stong *et al.*, 1985) or B1 cell line (Cohen *et al.*, 1991), both of which express CD19 and HLA Class I and carry the 4:11 translocation.

The RS4:11 cell line was established from bone marrow of a patient with t(4:11)-associated acute leukemia. Morphological, immunologic, and cytochemical characteristics of RS4:11 cells were found to be consistent with ALL. The cells are strongly positive for TdT. An in-depth analysis of RS4:11 revealed characteristics of both lymphoid and myeloid lineages.

The cells are rearranged for immunoglobulin heavy and k-chain genes, providing strong evidence for a commitment to B cell lineage. Although occasional heavy chain gene rearrangements have been noted in T cells and myeloid cells, light chain gene rearrangements have been restricted to the B cell lineage (Arnold *et al.*, 1983; Korsmeyer *et al.*, 1983; Ford *et al.*, 1983). The expression of B4 is additional support for B lineage classification, since within the hematopoietic system, this antigen is expressed very early in normal B cell ontogeny and is restricted to B lineage cells (Nadler *et al.*, 1983). Reactivity with BA-1, BA-2, and PI153/3 is consistent with B lineage classification because these MoAbs react with normal pre-B and B cells as well as with the vast majority of non-T ALL, although their binding cannot be considered to be definitive for lymphoid leukemias (LeBien *et al.*, 1983).

In addition to these lymphoid characteristics, RS4:11 cells bind 1G10, a mAb that reacts with granulocytic cells, some monocytes (Bernstein *et al.*, 1988), and CFU-GM precursor cells (Andrews *et al.*, 1983). Some RS4:11 cells weakly express the gp170,95/TA-1 antigen found on monocytic precursors (Andrews *et al.*, 1983) and peripheral blood monocytes (LeBein *et al.*, 1980). The ultrastructural detection of basophil/mast cell granules and peroxidase activity in a minor population of RS4:11 cells is supportive evidence of myeloid commitment. Similar basophil/mast cell granules have been detected in some cases of lymphoid blast crisis of chronic

myelogenous leukemia and in Philadelphia-positive ALL (Parkin *et al.*, 1982). The disappearance of this more differentiated subpopulation of RS4:11 suggests that these cells were at proliferative disadvantage or that the in vitro conditions could not support their phenotypic expression.

The monocyte-like phenotype of RS4:11 induced after TPA treatment is persuasive evidence for the myelomonocytic nature of RS4:11. Several laboratories have reported that TPA can induce human myeloid and lymphoid leukemic cells to more differentiated phenotypes that are primarily dictated by the differentiative potential of the target cells (Koeffler, 1983; LeBien *et al.*, 1982; Nadler *et al.*, 1982; Nagasawa *et al.*, 1980). In response to TPA (0.5 to 10.0 ng/mL), RS4:11 cells became reactive with TA-1, OKM1, and MCS2, became phagocytic, and showed greatly enhanced NSE activity in a pattern characteristic of monocytic cells. A subpopulation of treated cells became adherent, but this response resembled the weak adherence of certain TPA-treated lymphoid lines (Castagna *et al.*, 1979) more closely than the strong adherence displayed by treated myeloid lines, such as HL-60 and KG-1 (Koeffler, 1983; Goodwin *et al.*, 1984). Ultrastructurally, treated cells exhibited a monocytoid morphology.

The cell line B1 was established from bone marrow obtained from a 14-year-old child in first relapse. The patient's bone marrow sample at diagnosis and relapse contained over 95% malignant cells characterized by the t(4:11) (q21;q23) chromosomal translocation and biphenotypic expression of lymphoid and myeloid cell markers (often associated with this translocation).

The cell line was established by incubating leukemic cells (10^6/mL) in ∞-MEM containing 10% heat-inactivated fetal calf serum (FCS). After 8 weeks, the cells were cloned in semisolid methylcellulose and single colonies were isolated and expanded in liquid culture medium. The cell line established this way resembled the donor's leukemic cells. The karyotype of the line showed t (4:11) (q21; a23) in all metaphases. In addition, other chromosomal abnormalities, including trisomy 6,

der(1)t(1;8) (p36; q13), der(10)t(1;10)(q11; p15), were consistently observed in all metaphases. Cytochemical analysis showed a profile of periodic acid Schiff (PAS)-positive, acid phosphatase-positive, nonspecific esterase-positive, and Sudan black-negative staining. The leukemic cells lacked T- and B-cell markers (E-, sIg-, cIg-) and were CD10- and CD20-, but had undergone IgH(μ) gene rearrangement. Flow cytometric analysis showed that B1 cells expressed early pre-B-cell markers such as CD19+ and HLA-DR+. HLA-DR is coexpressed with My-9 (CD33), a marker of myeloid lineage on 20% of the cells. Other myeloid differentiation markers, such as My-7, Mo-1, and Mo-2, were undetectable on the surface of B1 cells.

These differentiation markers expressed on the B1 cell line are consistent with the early B and myeloid biphenotypic nature of the original bone marrow cells from this patient at relapse, and with previous reports of the association of the 4:11 translocation with biphenotypic leukemia.

All reactions were carried out at 4° C. Cells were counted and approximately 10^5 cells were aliquoted into polystyrene tubes. The cells were then incubated with FACS buffer (PBS containing 1% calf serum) for 20 minutes to block non-specific adherence of the antibodies. Cells were stained with the antibodies or scFv in a total volume of 200 μl for 20 minutes before being centrifuged and the supernatant discarded. Cells were then washed twice with FACS buffer before addition of 200 μl of biotinylated 25C1 antibody and streptavidin conjugated to phycoerythrin. The antibody was removed and the cells were washed again before addition of the anti HLA-class I antibody conjugated to FITC. After a final series of washes the cells were resuspended in PBS containing 0.4% paraformaldehyde. Fluorescence staining was measured by flow cytometry.

FACS analyses were used to evaluate the scFvs. The scFvs from B43 and 25C1 hybridomas (which are refered to as FVS191 (*F*ragment, *V*ariable, *S*ingle chain, anti CD*19*, number *1*) and FVS192, respectively, were able to inhibit the binding of FITC labeled 25C1 but not an anti HLA class I

monclonal antibody to cells that were CD19+, HLA class 1+ (Fig. 5). The scFv derived from BLy3 (FV S193) did not block the binding of the competing antibody. Also, binding to target cells could not be detected by biotinylating the scFv developed from this hybridoma and using this material with streptavidin labeled phycoerythrin (data not shown). The failure of BLy3 scFv to bind in these two assays suggests that the protein was not properly folded.

II. Scatchard analysis.

Iodine labelling of the proteins was accomplished using Iodobeads (Pierce) and the specific activity was determined. Beads were washed with iodination buffer, dried, and added to solution of carrier free Na125I (1 mCi/100 μg of protein) and allowed to react for five minutes. The reaction was stopped and the beads were washed. Gel filtration (Pharmacia PD5) was used to remove excess Na125I. TCA precipitation was carried out followed by determination of specific activity using standard calculations. Immunoreactive fractions were subsequently determined (with reagents generally in the range of 0.05). Scatchard analysis was determined using FACS buffer and labelled protein diluted serially in unlabelled protein to give a final concentration of 200 μg/mL. Iodinated protein was purified by Dowex or size exclusion column chromatography and the specific activity was calculated.

Due to the ability of FVS191 and FVS192 to specifically bind to cells that express the CD19 antigen we evaluated their affinity. Proteins were iodinated and used for Scatchard analysis as described in Materials and Methods. The results (Fig.6) demonstrated that the FVS191 had an K_a of $2x10^9$ M^{-1}. Although FVS192 was able to successfully compete with 25C1 binding to the CD19 antigen it did not demonstrate sufficient avidity of binding to be evaluated in Scatchard analysis and its Ka therefore could not be determined.

Example 4: Formation of dimers of Anti-CD19 Single-Chain Fv.

Single-chain Fv antibody fragments have the advantage of improved tumor penetration over intact antibody. Dimers of scFv may

possess higher binding constants and have potential as diagnostic or therapeutic agents.

To facilitate dimer formation, an additional cysteine residue was site-specifically inserted at the C-terminal of the scFv constructs of the present invention to form the scFv-cys. The scFv-cys proteins were isolated from bacterial inclusion bodies, reduced with guanidine, and refolded in redox buffer containing DTE and GSSG. Q-Sepharose-purified scFv-cys proteins were treated with 2 mM DTT. The DTT was removed using a Pharmacia PD10 column. Disulfide bonds between C-terminal cysteins were formed by air oxidation. Dimer formation of both B43 scFv-cys and 25C1 scFv-cys was confirmed by reducing and non-reducing SDS-PAGE. The scFv without C-terminal cysteine did not form covalently-linked dimers under these conditions, indicating that these dimers were indeed formed by the specific disulfide linkage between C-terminal cysteines.

Example 5: Animal Studies

Leukemia is likely to be successfully treated using radiolabled anti-CD19 scFv because it is radiosensitive and there is ready access of antibody to the marrow space. Clinical studies have shown that iodine-labled antiferritin antibodies provided symptomatic relief to 77% patients with refractory Hodgkin disease and produced objective tumor regression in 40% of patients. In another clinical trail, when radiolabled anti-CD33 and -35 antibodies were used in combination with high a dose of cyclophosphamide, an overall of 19% complete remissions and 75% partial remissions were achieved for 210 evaluable patients with hematologic malignancies. The major side effect associated with the use of iodine-labled antibodies was reported to be thrombocytopenia, which occurred more frequently when the dose of iodine used was greater than 200 mCi/patient (see review by Grossbard *et al.*, 1992).

Radiolabled antibodies kill target cells by by-stander effect. Internalization of radiolabled antibodies is probably not desirable. It has shown that internalized radiolabled antibodies had a much shorter

retention time and a faster rate of deiodination, which would dramatically reduce the efficacy of the therapeutic values of the antibodies (Richard et al., 1992). The single chain antibodies have the advantages of being small, with relatively high affinity toward the antigens and not being internalized by the target cells.

A. *Preparation of FVS 191 and FVS 192 single chain antibody*

The antibody is expressed in *Escherichia coli* as inclusion bodies. The inclusion bodes are denatured, refolded, and purified by FPLC chromatography. Since endotoxin contents of the antibody is high, it must be removed before being used in animals. Endotoxin is removed by affinity chromatography (a kit is commercially available). The amount of endotoxin in the antibody preparation is monitored by the Limulus Amebocyte Lysate Assay (Biowhittater Inc., Walkersville, MD). According to the US standard, the endotoxin contents in the final antibody preparation must be reduced to <15 endotoxin unit (EU, 1 EU = 0.5 ng/ml).

B. *Iodination*

The single chain antibody is labeled with Na ^{131}I using a Idogen kit (Pierce, Rockford, IL). The ratio of Idogen to antibody is adjusted to approximately 100ug:1mg as described by Badger et al. (1985). The labeled antibody will be separated from free ^{131}I by gel filtration. The labeling efficiency and specific activity will be determined by cyclic anhydride method (Hantowich *et al.*, 1983). A specific activity of 1.0Ci/g or less should be suitable for the experiments. The same amounts of whole monoclonal antibody and Fab of an unrelated antibody should be labeled with ^{131}I the same way to serve as controls.

C. *Determination of immunoreactivity.*

Immunoreactivity is defined as percentage of counts that are able to bind at antigen excess. Briefly, a serial dilution of target cells (CD19+, 10^{6-7}/ml will be incubated with labeled antibody (4-5 ng/ml) for 1 h at RT. Cells are centrifuged and supernatant radioactivity is counted. Immunoreactivity will be determined by Lineweaver-Burk analysis. Avidity of the antibody will be determined by incubation fixed amounts of

cells (10^5/ml) with a serial dilutions of labeled antibody for 1 h at RT. Cells are washed and the cell pellet radioactivity is used to calculate the avidity (association constant and the number of binding site per cell).

D. *In vitro measurement of single chain Fv metabolism.*

This experiment determines the rate at which the labeled antibody is internalized and degraded. The target cells are incubated with labeled antibodies (scFv and whole Mab, 5 ng/10^6 cells) in a volume of 100 ul for 45 min on ice. The cells are washed and cultured at 37C. Aliquots of the incubation mixture are assayed for cell associated and supernatant radioactivity at 0, 1, 4, 10 and 24 h. the percentage of TCA precipitated radioactivity will be used for calculating the rate of internalization and intracellular metabolism of the labeled antibody.

1. *Pharmacokinetic Studies*

Pharmacokinetic studies are carried out by injecting labeled single chain antibody into a group of 4 BALB/c mice via the tail vein. Blood samples are collected at various time intervals. Radioactivities associated with the blood samples will be determined and T alpha 1/2 and T beta 1/2 of the single chain antibody will be calculated by computer simulation. As a control, the parental monoclonal antibody is labeled and injected into the mice as described above. Biodistribution is performed with paired labeling, *e.g.* the single chain antibody will be labeled with ^{131}I and the controlled antibody labeled with ^{125}I. In our laboratory, anti CD19 scFv, FVS 191, has been successfully labled with ^{125}I and used in immunochemistry and pharmacokinetics studies. Using current protocal, this scFv can be readily labled with ^{125}I with a specific activity of 2.4 mci/mg. The immunoreactivity of the labled antibody was 55%. FVS 191 is more resistant to labeling damage than intact antibody. Results of Scatchard analysis showed that the affinity of FVS 191 toward CD19 antigen was 7.2 X 10^8 M^{-1}. This value is about four fold higher than its parent monoclonal antibody (1.93 X 10^8 M^{-1}), suggesting that scFv may be a better targeting reagent than intact antibody. The observation that scFv showed higher affinity than its parent intact monoclonal antibody is

consistent with the findings of others. Data from pharmacokinetic studies in BALB/c mice showed that FVS 191 had a $T_{1/2}A$ and $T_{1/2}B$ of 2.5 min and 3.7 h respectively. In comparison, the intact monoclonal antibody had a $T_{1/2}A$, and $T_{1/2}B$ = 7.2 min. and 57.1 h. In summary, the high specific immunoreactivity, high affinity and the rapid blood clearance of anti FVS 191 makes it an excellent candidate for use in cancer therapy.

2. *Biodistribution Studies*

A mixture of equivalent amounts of specific antibody and control antibody with varied concentrations is injected i.v. into a group of 4 mice with human leukemia xenografts. The animals are sacrificed at 1, 24 and 48 h after the injection. Samples of blood, tumor, lung, spleen and kidney are weighed and counted in a gamma counter. The percentage of injected dose per gram of tissue (%ID/g) for each isotope is calculated. For dose escalation studies a single labeling (^{131}I) will be performed to determine the proper dose range for subsequent animal survival tests.

E. *Demonstration of Therapeutic Efficacy*

Two types of leukemia animal models are used in the experiments — *e.g.* acute human leukemia (B1 or RS4:11 cell) in SCID mice or human acute leukemia xenograft tumor model in SCID or athymic BALB/c mice. The human leukemia SCID model has been well established in this laboratory and should be readily available for the experiments. The xenograft tumor model is established by injecting human leukemia cells (4-5 X 10^7 in 0.2 ml PBS) into flanks of the mice as described by Richard et al, 1992). A palpable tumor module of 0.5-1.0 cm should be detected 8-10 days after the tumor cell injection. A single infusion (i.v) of various concentrations (low, medium and high) of radiolabled antibody is given to a group of 4 animals. The same amount of controlled antibody labeled with ^{131}I are treated the same way. The percentage of survival will be recorded up to 50 days. For animals with xenografts, regression of tumors will be recorded. The definition of complete, and partial regressions needs to be defined.

1. *Therapeutic results*

Since FVS 191 and FVS 192 single chain antibody are specific for CD19+ cells, radiolabled antibody should show significant target cell killing effect in comparison to control antibody. Complete or partial tumor regression after radiolabled antibody treatment is expected. Due to its small size, single chain antibody is expected to penetrate the tumor more efficiently and show better results as compared to labeled whole MAb.

2. *Therapy of Human B Cell Cancer (leukemia and lymphoma)*

The anti CD19 scFv will be conjugated to ^{131}I as described in animal therapy studies. Initial human trials will focus on pharmacokinetic and biodistribution studies.

Anti-CD19 antibodies have been effective for the treatment of human B cell leukemias or lymphomas when conjugated to toxins, *e.g.*, ricin or pokeweed antiviral protein (Vitetta, *et al.*, Uckun, *et al.*). B cell antibodies other than CD19 (*e.g.*, anti–CD29) have been effective when linked to radioisotopes, *e.g.* ^{131}I. Experience to date indicates that anti–CD19 FVS 191 and FVS 192 are not internalized by the cell after binding and thus these scFv should be effective as radioimmunoconjugates which should remain on the cell surface for optimal stability and cell killing. The anti-CD19 scFv will have very efficient biodistribution and tissue penetration based on the small size and short half life. As noted earlier, FVS 191 has a $T_{1/2}$ of 2.5 minutes in the alpha phase and $T_{1/2}$ of 3.7 hour in the beta phase. Thus, the rapid clearance combined should allow excellent killing of essentially all B cell leukemias and lymphomas (99% of which bear CD19). The small size of the ^{131}I scFv should allow excellent killing in marrow lymph nodes and extromedullary sites which often serve as sanctuaries for leukemia and lymphoma cells.

REFERENCES

The references listed below, and all references cited herein, are incorporated by reference to the extent that they supplement, explain, provide a backgrond for, or teach methodology, techniques and/or compositions employed herein.

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Brief Description of Sequences

The following list briefly identifies the sequences discussed in the specification and claims:

SEQ ID NO:1	5′ Oligonucleotide used for PCR of heavy chain variable region
SEQ ID NO:2	3′ Oligonucleotide used for PCR of heavy chain variable region
SEQ ID NO:3	3′ Oligonucleotide used for PCR of heavy chain constant region
SEQ ID NO:4	5′ Oligonucleotide used for PCR of light chain variable region
SEQ ID NO:5	3′ Oligonucleotide used for PCR of light chain variable region
SEQ ID NO:6	3′ Oligonucleotide used for PCR of light chain constant region
SEQ ID NO:7	Linker DNA between variable light and heavy chain regions
SEQ ID NO:8	cDNA sequence of B43 Heavy chain
SEQ ID NO:9	cDNA sequence of SJ25C1 Heavy chain
SEQ ID NO:10	cDNA sequence of BLY3 Heavy chain

SEQ ID NO:11 cDNA sequence of B43 Light chain

SEQ ID NO:12 cDNA sequence of SJ25C1 Light chain

SEQ ID NO:13 cDNA sequence of BLY3 Light chain

SEQ ID NO:14 Protein sequence of B43 Heavy chain

SEQ ID NO:15 Protein sequence of SJ25C1 Heavy chain

SEQ ID NO:16 Protein sequence of BLY3 Heavy chain

SEQ ID NO:17 Protein sequence of B43 Light chain

SEQ ID NO:18 Protein sequence of SJ25C1 Light chain

SEQ ID NO:19 Protein sequence of BLY3 Light chain

SEQ ID NO:20 Protein sequence of single chain B43 antibody

SEQ ID NO:21 Protein sequence of single chain SJ25C1 antibody

SEQ ID NO:22 Protein sequence of single chain BLY3 antibody

SEQ ID NO:23 cDNA sequence of single chain B43 antibody

SEQ ID NO:24 cDNAsequence of single chain SJ25C1 antibody

SEQ ID NO:25 cDNA sequence of single chain BLY3 antibody

SEQ ID NO:26 Protein sequence of modified single chain B43 antibody

SEQ ID NO:27 Protein sequence of modified single chain SJ25C1 antibody

SEQ ID NO:28 Protein sequence of the dimer single chain B43 antibody

SEQ ID NO:29 Protein sequence of the dimer single chain SJ25C1 antibody

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Bejcek, Bruce E.
Wang, Duo
Uckun, Fatih M.
Kersey, John H.

(ii) TITLE OF INVENTION:

IMMUNOCONJUGATES FROM SINGLE-CHAIN VARIABLE REGION FRAGMENTS OF ANTI-CD19 ANTIBODIES

(iii) NUMBER OF SEQUENCES: 29

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Patterson & Keough, P.A.

(B) STREET: 527 Marquette Avenue South, Suite 1200

(C) CITY: Minneapolis

(D) STATE: Minnesota

(E) COUNTRY: USA

(F) ZIP: 55455

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy diskette, 3.5 inch

(B) COUMPUTER: Apple Macintosh

(C) OPERATING SYSTEM: Apple Macintosh System 7.0

(D) SOFTWARE: WordPerfect 3.0 for Macintosh

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Daniel F. Coughlin, Esq.

(B) REGISTRATION NUMBER: 36,111

(C) REFERENCE/DOCKET NUMBER:

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 612/349-5759

(B) TELEFAX: 612/349-9266

(2) INFORMATION FOR SEQ ID NO:1

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(x) PUBLICATION INFORMATION:

(A) AUTHORS: *

(B) TITLE: *

(C) JOURNAL: *

(D) VOLUME: *

(E) ISSUE: *

(F) PAGES: *

(G) DATE: *

(H) DOCUMENT NUMBER: *

(I) FILING DATE: *

(J) PUBLICATION DATE: *

(K) RELEVANT RESIDUES: *

(xi) A SEQUENCE DESCRIPTION: SEQ ID NO: 1:

AGGTCCAGCT GCTCGAGTCT GG 22

(3) INFORMATION FOR SEQ ID NO:2

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(x) PUBLICATION INFORMATION:

(A) AUTHORS: *

(B) TITLE: *

(C) JOURNAL: *

(D) VOLUME: *

(E) ISSUE: *

(F) PAGES: *

(G) DATE: *

(H) DOCUMENT NUMBER: *

(I) FILING DATE: *

(J) PUBLICATION DATE: *

(K) RELEVANT RESIDUES: *

(xi) A SEQUENCE DESCRIPTION: SEQ ID NO: 2:

TGAGGAGACG GTGACCGTGT CCCTTGGCCC CAG 33

(4) INFORMATION FOR SEQ ID NO:3

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(x) PUBLICATION INFORMATION:

(A) AUTHORS: *

(B) TITLE: *

(C) JOURNAL: *

(D) VOLUME: *

(E) ISSUE: *

(F) PAGES: *

(G) DATE: *

(H) DOCUMENT NUMBER: *

(I) FILING DATE: *

(J) PUBLICATION DATE: *

(K) RELEVANT RESIDUES: *

(xi) A SEQUENCE DESCRIPTION: SEQ ID NO: 3:

AGGCTTACTA GTACAATCCC TGGGCACAAT 30

(5) INFORMATION FOR SEQ ID NO:4

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(x) PUBLICATION INFORMATION:

(A) AUTHORS: *

(B) TITLE: *

(C) JOURNAL: *

(D) VOLUME: *

(E) ISSUE: *

(F) PAGES: *

(G) DATE: *

(H) DOCUMENT NUMBER: *

(I) FILING DATE: *

(J) PUBLICATION DATE: *

(K) RELEVANT RESIDUES: *

(xi) A SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CGCGGATCCA GTTCCGAGCT CGTGCTCACC CAGTCTCCA 39

(6) INFORMATION FOR SEQ ID NO:5

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(x) PUBLICATION INFORMATION:

(A) AUTHORS: *

(B) TITLE: *

(C) JOURNAL: *

(D) VOLUME: *

(E) ISSUE: *

(F) PAGES: *

(G) DATE: *

(H) DOCUMENT NUMBER: *

(I) FILING DATE: *

(J) PUBLICATION DATE: *

(K) RELEVANT RESIDUES: *

(xi) A SEQUENCE DESCRIPTION: SEQ ID NO: 5:

GAAGATCTAC GTTTTATTTC CAGCTTGGTC CC 32

(7) INFORMATION FOR SEQ ID NO:6

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 34

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(x) PUBLICATION INFORMATION:

(A) AUTHORS: *

(B) TITLE: *

(C) JOURNAL: *

(D) VOLUME: *

(E) ISSUE: *

(F) PAGES: *

(G) DATE: *

(H) DOCUMENT NUMBER: *

(I) FILING DATE: *

(J) PUBLICATION DATE: *

(K) RELEVANT RESIDUES: *

(xi) A SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GCGCCGTCTA GAATTAACAC TCATTCCTGT TGAA 34

(8) INFORMATION FOR SEQ ID NO:7

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 45

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double stranded

(D) TOPOLOGY: linear

(x) PUBLICATION INFORMATION:

(A) AUTHORS: *

(B) TITLE: *

(C) JOURNAL: *

(D) VOLUME: *

(E) ISSUE: *

(F) PAGES: *

(G) DATE: *

(H) DOCUMENT NUMBER: *

(I) FILING DATE: *

(J) PUBLICATION DATE: *

(K) RELEVANT RESIDUES: *

(xi) A SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GGA GGC GGT GGC TCG GGC GGT GGC GGC TCG GGT GGC GGC GGA TCC 45
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
5 10 15

(9) INFORMATION FOR SEQ ID NO:8

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 351

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double stranded

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(A) DESCRIPTION: Heavy chain B43 DNA

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: Anti CD-19 hybridomas

(B) CLONE: B43 cell line

(x) PUBLICATION INFORMATION:

(A) AUTHORS: *

(B) TITLE: *

(C) JOURNAL: *

(D) VOLUME: *

(E) ISSUE: *

(F) PAGES: *

(G) DATE: *

(H) DOCUMENT NUMBER: *

(I) FILING DATE: *

(J) PUBLICATION DATE: *

(K) RELEVANT RESIDUES: *

(xi) A SEQUENCE DESCRIPTION: SEQ ID NO: 8:

```
CTC GAG TCT GGG GCT GAG CTG GTG AGG CCT GGG TCC TCA GTG AAG ATT  48
Leu Glu Ser Gly Ala Glu Leu Val Arg Pro Gly Ser Ser Val Lys Ile
                5                  10                  15

TCC TGC AAG GCT TCT GGC TAT GCA TTC AGT AGC TAC TGG ATG AAC TGG  96
Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr Trp Met Asn Trp
            20                  25                  30

GTG AAG CAG AGG CCT GGA CAG GGT CTT GAG TGG ATT GGA CAG ATT TGG  144
Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Gln Ile Trp
        35                  40                  45

CCT GGA GAT GGT GAT ACT AAC TAC AAT GGA AAG TTC AAG GGT AAA GCC  192
Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys Gly Lys Ala
    50                  55                  60

ACT CTG ACT GCA GAC GAA TCC TCC AGC ACA GCC TAC ATG CAA CTC AGC  240
Thr Leu Thr Ala Asp Glu Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser
65                  70                  75                  80

AGC CTA CGA TCT GAG GAC TCT GCG GTC TAT TCT TGT GCA AGA CGG GAG  288
Ser Leu Arg Ser Glu Asp Ser Ala Val Tyr Ser Cys Ala Arg Arg Glu
                85                  90                  95
```

ACT ACG ACG GTA GGC CGT TAT TAC TAT GCT ATG GAC TAC TGG GGC CAA 336
Thr Thr Thr Val Gly Arg Tyr Tyr Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110

GGG ACC ACG GTC ACC 351
Gly Thr Thr Val Thr
115

(10) INFORMATION FOR SEQ ID NO:9

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 345

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double stranded

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(A) DESCRIPTION: Heavy chain SJ25C1 DNA

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: Anti CD-19 hybridomas

(B) CLONE: SJ25C1 cell line

(x) PUBLICATION INFORMATION:

(A) AUTHORS: *

(B) TITLE: *

(C) JOURNAL: *

(D) VOLUME: *

(E) ISSUE: *

(F) PAGES: *

(G) DATE: *

(H) DOCUMENT NUMBER: *

(I) FILING DATE: *

(J) PUBLICATION DATE: *

(K) RELEVANT RESIDUES: *

(xi) A SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```
CTC GAG TCT GGG GCT GAG CTG GTG AGG CCT GGG TCC TCA GTG AAG ATT  48
Leu Glu Ser Gly Ala Glu Leu Val Arg Pro Gly Ser Ser Val Lys Ile
                5                  10                  15

TCC TGC AAG GCT TCT GGC TAT GCA TTC AGT AGC TAC TGG ATG AAC TGG  96
Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr Trp Met Asn Trp
            20                  25                  30
GTG AAG CAG AGG CCT GGA CAG GGT CTT GAG TGG ATT GGA CAG ATT TAT  144
Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Gln Ile Tyr
        35                  40                  45

CCT GGA GAT GGT GAT ACT AAC TAC AAT GGA AAG TTC AAG GGT CAA GCC  192
Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys Gly Gln Ala
    50                  55                  60

ACA CTG ACT GCA GAC AAA TCC TCC AGC ACA GCC TAC ATG CAG CTC AGC  240
Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser
65                  70                  75                  80
```

GGC CTA ACA TCT GAG GAC TCT GCG GTC TAT TCT TGT GCA AGA AAG ACC 288
Gly Leu Thr Ser Glu Asp Ser Ala Val Tyr Ser Cys Ala Arg Lys Thr
85 90 95

ATT AGT TCG GTA GTA GAT TTC TAC TTT GAC AAC TGG GGC CAA GGG ACC 336
Ile Ser Ser Val Val Asp Phe Tyr Phe Asp Asn Trp Gly Gln Gly Thr
100 105 110

ACG GTC ACC 345
Thr Val Thr
115

(11) INFORMATION FOR SEQ ID NO:10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 342

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double stranded

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(A) DESCRIPTION: Heavy chain BLY3 DNA

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: Anti CD-19 hybridomas

(B) CLONE: BLY3 cell line

(x) PUBLICATION INFORMATION:

(A) AUTHORS: *

(B) TITLE: *

(C) JOURNAL: *

(D) VOLUME: *

(E) ISSUE: *

(F) PAGES: *

(G) DATE: *

(H) DOCUMENT NUMBER: *

(I) FILING DATE: *

(J) PUBLICATION DATE: *

(K) RELEVANT RESIDUES: *

(xi) A SEQUENCE DESCRIPTION: SEQ ID NO: 10:

```
CTC GAG TCT GGG GCT GAG CTG GTG AGG CCT GGG GCC TCA GTG AAG ATT  48
Leu Glu Ser Gly Ala Glu Leu Val Arg Pro Gly Ala Ser Val Lys Ile
                5                  10                  15

TCC TGC AAA GCT TCT GGC TAC GCA TTC AGT AGC TCT TGG ATG AAC TGG  96
Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Ser Trp Met Asn Trp
            20                  25                  30

GTG AAG CAG AGG CCT GGA CAG GGT CTT GAG TGG ATT GGA CGG ATT TAT  144
Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Arg Ile Tyr
        35                  40                  45

CCT GGA GAT GGA GAT ACT AAC TAC AAT GGA AAG TTC AAG GAA GCG GCC  192
Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys Glu Ala Ala
```

50 55 60

ACA CTG ACT GCA GAC AAA TCC TCC AGC ACA GCG TAC ATG CAG CTC AGC 240
Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser
65 70 75 80

AGC CTG ACC TCT GTG GAC TCT GCG GTC TAT TCT TGT GCA AGA TCG GAG 288
Ser Leu Thr Ser Val Asp Ser Ala Val Tyr Ser Cys Ala Arg Ser Glu
85 90 95

TAT TGG GGT AAC TAC TGG GCT ATG GAC TAC TGG GGC CAA GGG ACC ACG 336
Tyr Trp Gly Asn Tyr Trp Ala Met Asp Tyr Trp Gly Gln Gly Thr Thr
100 105 110

GTC ACC 342
Val Thr

(12) INFORMATION FOR SEQ ID NO:11

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 342

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single stranded

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(A) DESCRIPTION: Light chain B43 DNA

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: Anti CD-19 hybridomas

(B) CLONE: B43 cell line

(x) PUBLICATION INFORMATION:

(A) AUTHORS: *

(B) TITLE: *

(C) JOURNAL: *

(D) VOLUME: *

(E) ISSUE: *

(F) PAGES: *

(G) DATE: *

(H) DOCUMENT NUMBER: *

(I) FILING DATE: *

(J) PUBLICATION DATE: *

(K) RELEVANT RESIDUES: *

(xi) A SEQUENCE DESCRIPTION: SEQ ID NO: 11:

```
GAG CTC GTG CTC ACC CAG TCT CCA GCT TCT TTG GCT GTG TCT CTA GGG  48
Glu Leu Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
                5                  10                  15

CAG AGG GCC ACC ATC TCC TGC AAG GCC AGC CAA AGT GTT GAT TAT GAT  96
Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp
            20                  25                  30

GGT GAT AGT TAT TTG AAC TGG TAC CAA CAG ATT CCA GGA CAG CCA CCC  144
Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro
        35                  40                  45
```

```
AAA CTC CTC ATC TAT GAT GCA TCC AAT CTA GTT TCT GGG ATC CCA CCC  192
Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser Gly Ile Pro Pro
    50                  55                  60

AGG TTT AGT GGC AGT GGG TCT GGG ACA GAC TTC ACC CTC AAC ATC CAT  240
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65                  70                  75                  80

CCT GTG GAG AAG GTG GAT GCT GCA ACC TAT CAC TGT CAG CAA AGT ACT  288
Pro Val Glu Lys Val Asp Ala Ala Thr Tyr His Cys Gln Gln Ser Thr
                85                  90                  95

GAG GAT CCG TGG ACG TTC GGT GGA GGG ACC AAG CTG GAA ATA AAA CGT  336
Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
            100                 105                 110

AGA TCT                                                         342
Arg Ser
```

(13) INFORMATION FOR SEQ ID NO:12

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 333

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double stranded

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: cDNA

(A) DESCRIPTION: Light chain SJ25C1 DNA

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: Anti CD-19 hybridomas

(B) CLONE: SJ25C1 cell line

(x) PUBLICATION INFORMATION:

(A) AUTHORS: *

(B) TITLE: *

(C) JOURNAL: *

(D) VOLUME: *

(E) ISSUE: *

(F) PAGES: *

(G) DATE: *

(H) DOCUMENT NUMBER: *

(I) FILING DATE: *

(J) PUBLICATION DATE: *

(K) RELEVANT RESIDUES: *

(xi) A SEQUENCE DESCRIPTION: SEQ ID NO: 12:

```
GAG CTC GTG CTC ACC CAG TCT CCA AAA TTC ATG TCC ACA TCA GTA GGA  48
Glu Leu Val Leu Thr Gln Ser Pro Lys Phe Met Ser Thr Ser Val Gly
                5                  10                  15

GAC AGG GTC AGC GTC ACC TGC AAG GCC AGT CAG AAT GTG GGT ACT AAT  96
Asp Arg Val Ser Val Thr Cys Lys Ala Ser Gln Asn Val Gly Thr Asn
            20                  25                  30
```

```
GTA GCC TGG TAT CAA CAG AAA CCA GGA CAA TCT CCT AAA CCA CTG ATT  144
Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Pro Leu Ile
        35                  40                  45

TAC TCG GCA ACC TAC CGG AAC AGT GGA GTC CCT GAC CGC TTC ACA GGC  192
Tyr Ser Ala Thr Tyr Arg Asn Ser Gly Val Pro Asp Arg Phe Thr Gly
    50                  55                  60

AGT GGA TCT GGG ACA GAT TTC ACT CTC ACC ATC ACT AAC GTG CAG TCT  240
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Asn Val Gln Ser
65                  70                  75                  80

AAA GAC TTG GCA GAC TAT TTC TAT TTC TGT CAA TAT AAC AGG TAT CCG  288
Lys Asp Leu Ala Asp Tyr Phe Tyr Phe Cys Gln Tyr Asn Arg Tyr Pro
                85                  90                  95

TAC ACG TCC GGA GGG GGG ACC AAG CTG GAA ATA AAA CGT AGA TCT      333
Tyr Thr Ser Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Arg Ser
            100                 105                 110
```

(14) INFORMATION FOR SEQ ID NO:13

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 342

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double stranded

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(A) DESCRIPTION: Light chain BLY3 DNA

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: Anti CD-19 hybridomas

(B) CLONE: BLY3 cell line

(x) PUBLICATION INFORMATION:

(A) AUTHORS: *

(B) TITLE: *

(C) JOURNAL: *

(D) VOLUME: *

(E) ISSUE: *

(F) PAGES: *

(G) DATE: *

(H) DOCUMENT NUMBER: *

(I) FILING DATE: *

(J) PUBLICATION DATE: *

(K) RELEVANT RESIDUES: *

(xi) A SEQUENCE DESCRIPTION: SEQ ID NO: 13:

GAG CTC GTG CTC ACC CAG TCT CCA GCT TCT TTG GCT GTG TCT CTA GGG 48
Glu Leu Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
5 10 15

```
CAG AGG GCC ACC ATC TCC TGC AGA GCC AGC CAG AGT GTT GAT AAT TAT  96
Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Gln Ser Val Asp Asn Tyr
            20                  25                  30

GGC ATT AGT TTT ATG AAC TGG TTC CAA CAG AAA CCA GGA CAG CCA CCC 144
Gly Ile Ser Phe Met Asn Trp Phe Gln Gln Lys Pro Gly Gln Pro Pro
        35                  40                  45

AAA CTC CTC ATC TAT GCT GCA TCC AAC CAA GGA TCC GGG GTC CCT GCC 192
Lys Leu Leu Ile Tyr Ala Ala Ser Asn Gln Gly Ser Gly Val Pro Ala
    50                  55                  60

AGG TTT AGT GGC AGT GGG TCT GGG ACA GAC TTC AGC CTC AAC ATC CAT 240
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu Asn Ile His
65                  70                  75                  80

CCT ATG GAG GAG GAT GAT ACT GCA ATG TAT TTC TGT CAG CAA AGT AAG 288
Pro Met Glu Glu Asp Asp Thr Ala Met Tyr Phe Cys Gln Gln Ser Lys
                85                  90                  95

GAG GTT CCT CGG ACG TTC GGT GGA GGG ACC AAG CTG GAA ATA AAA CGT 336
Glu Val Pro Arg Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
            100                 105                 110

AGA TCT                                                         342
Arg Ser
```

(15) INFORMATION FOR SEQ ID NO:14

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 117

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: protein

(A) DESCRIPTION: Heavy chain B43 protein

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: Anti CD-19 hybridomas

(B) CLONE: B43 cell line

(x) PUBLICATION INFORMATION:

(A) AUTHORS: *

(B) TITLE: *

(C) JOURNAL: *

(D) VOLUME: *

(E) ISSUE: *

(F) PAGES: *

(G) DATE: *

(H) DOCUMENT NUMBER: *

(I) FILING DATE: *

(J) PUBLICATION DATE: *

(K) RELEVANT RESIDUES: *

(xi) A SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Leu Glu Ser Gly Ala Glu Leu Val Arg Pro Gly Ser Ser Val Lys Ile
5 10 15

Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr Trp Met Asn Trp
20 25 30

Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Gln Ile Trp
35 40 45

Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys Gly Lys Ala
50 55 60

Thr Leu Thr Ala Asp Glu Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser
65 70 75 80

Ser Leu Arg Ser Glu Asp Ser Ala Val Tyr Ser Cys Ala Arg Arg Glu
85 90 95

Thr Thr Thr Val Gly Arg Tyr Tyr Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Thr Val Thr
115

(16) INFORMATION FOR SEQ ID NO:15

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 115

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: protein

(A) DESCRIPTION: Heavy chain SJ25C1 protein

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: Anti CD-19 hybridomas

(B) CLONE: SJ25C1 cell line

(x) PUBLICATION INFORMATION:

(A) AUTHORS: *

(B) TITLE: *

(C) JOURNAL: *

(D) VOLUME: *

(E) ISSUE: *

(F) PAGES: *

(G) DATE: *

(H) DOCUMENT NUMBER: *

(I) FILING DATE: *

(J) PUBLICATION DATE: *

(K) RELEVANT RESIDUES: *

(xi) A SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Leu Glu Ser Gly Ala Glu Leu Val Arg Pro Gly Ser Ser Val Lys Ile
5 10 15

Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr Trp Met Asn Trp
20 25 30

Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Gln Ile Tyr
35 40 45

Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys Gly Gln Ala
50 55 60

Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser
65 70 75 80

Gly Leu Thr Ser Glu Asp Ser Ala Val Tyr Ser Cys Ala Arg Lys Thr
85 90 95

Ile Ser Ser Val Val Asp Phe Tyr Phe Asp Asn Trp Gly Gln Gly Thr
100 105 110

Thr Val Thr
115

(17) INFORMATION FOR SEQ ID NO:16

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 114

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: protein

(A) DESCRIPTION: Heavy chain BLY3 protein

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: Anti CD-19 hybridomas

(B) CLONE: BLY3 cell line

(x) PUBLICATION INFORMATION:

(A) AUTHORS: *

(B) TITLE: *

(C) JOURNAL: *

(D) VOLUME: *

(E) ISSUE: *

(F) PAGES: *

(G) DATE: *

(H) DOCUMENT NUMBER: *

(I) FILING DATE: *

(J) PUBLICATION DATE: *

(K) RELEVANT RESIDUES: *

(xi) A SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Leu Glu Ser Gly Ala Glu Leu Val Arg Pro Gly Ala Ser Val Lys Ile
5 10 15

Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Ser Trp Met Asn Trp
20 25 30

Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Arg Ile Tyr
35 40 45

Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys Glu Ala Ala
50 55 60

Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser
65 70 75 80

Ser Leu Thr Ser Val Asp Ser Ala Val Tyr Ser Cys Ala Arg Ser Glu
85 90 95

Tyr Trp Gly Asn Tyr Trp Ala Met Asp Tyr Trp Gly Gln Gly Thr Thr
100 105 110

Val Thr

(18) INFORMATION FOR SEQ ID NO:17

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 114

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: protein

(A) DESCRIPTION: Light chain B43 protein

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: Anti CD-19 hybridomas

(B) CLONE: B43 cell line

(x) PUBLICATION INFORMATION:

(A) AUTHORS: *

(B) TITLE: *

(C) JOURNAL: *

(D) VOLUME: *

(E) ISSUE: *

(F) PAGES: *

(G) DATE: *

(H) DOCUMENT NUMBER: *

(I) FILING DATE: *

(J) PUBLICATION DATE: *

(K) RELEVANT RESIDUES: *

(xi) A SEQUENCE DESCRIPTION: SEQ ID NO: 17:

Glu Leu Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
5 10 15

Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp
20 25 30

Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro
35 40 45

Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser Gly Ile Pro Pro
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His

65 70 75 80

Pro Val Glu Lys Val Asp Ala Ala Thr Tyr His Cys Gln Gln Ser Thr
85 90 95

Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
100 105 110

Arg Ser

(19) INFORMATION FOR SEQ ID NO:18

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 111

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: protein

(A) DESCRIPTION: Light chain SJ25C1 protein

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: Anti CD-19 hybridomas

(B) CLONE: SJ25C1 cell line

(x) PUBLICATION INFORMATION:

(A) AUTHORS: *

(B) TITLE: *

(C) JOURNAL: *

(D) VOLUME: *

(E) ISSUE: *

(F) PAGES: *

(G) DATE: *

(H) DOCUMENT NUMBER: *

(I) FILING DATE: *

(J) PUBLICATION DATE: *

(K) RELEVANT RESIDUES: *

(xi) A SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Glu Leu Val Leu Thr Gln Ser Pro Lys Phe Met Ser Thr Ser Val Gly
5 10 15

Asp Arg Val Ser Val Thr Cys Lys Ala Ser Gln Asn Val Gly Thr Asn
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Pro Leu Ile
35 40 45

Tyr Ser Ala Thr Tyr Arg Asn Ser Gly Val Pro Asp Arg Phe Thr Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Asn Val Gln Ser
65 70 75 80

Lys Asp Leu Ala Asp Tyr Phe Tyr Phe Cys Gln Tyr Asn Arg Tyr Pro
85 90 95

Tyr Thr Ser Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Arg Ser
100 105 110

(20) INFORMATION FOR SEQ ID NO:19

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 114

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: protein

(A) DESCRIPTION: Light chain BLY3 protein

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: Anti CD-19 hybridomas

(B) CLONE: BLY3 cell line

(x) PUBLICATION INFORMATION:

(A) AUTHORS: *

(B) TITLE: *

(C) JOURNAL: *

(D) VOLUME: *

(E) ISSUE: *

(F) PAGES: *

(G) DATE: *

(H) DOCUMENT NUMBER: *

(I) FILING DATE: *

(J) PUBLICATION DATE: *

(K) RELEVANT RESIDUES: *

(xi) A SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Glu Leu Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
5 10 15

Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Gln Ser Val Asp Asn Tyr
20 25 30

Gly Ile Ser Phe Met Asn Trp Phe Gln Gln Lys Pro Gly Gln Pro Pro
35 40 45

Lys Leu Leu Ile Tyr Ala Ala Ser Asn Gln Gly Ser Gly Val Pro Ala
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu Asn Ile His
65 70 75 80

Pro Met Glu Glu Asp Asp Thr Ala Met Tyr Phe Cys Gln Gln Ser Lys
85 90 95

Glu Val Pro Arg Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
100 105 110

Arg Ser

(21) INFORMATION FOR SEQ ID NO:20

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 246

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: protein

(A) DESCRIPTION: single chain B43 protein

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: Anti CD-19 hybridomas

(B) CLONE: B43 cell line

(x) PUBLICATION INFORMATION:

(A) AUTHORS: *

(B) TITLE: *

(C) JOURNAL: *

(D) VOLUME: *

(E) ISSUE: *

(F) PAGES: *

(G) DATE: *

(H) DOCUMENT NUMBER: *

(I) FILING DATE: *

(J) PUBLICATION DATE: *

(K) RELEVANT RESIDUES: *

(xi) A SEQUENCE DESCRIPTION: SEQ ID NO: 20:

Leu Glu Ser Gly Ala Glu Leu Val Arg Pro Gly Ser Ser Val Lys Ile
5 10 15

Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr Trp Met Asn Trp
20 25 30

Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Gln Ile Trp
35 40 45

Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys Gly Lys Ala
50 55 60

Thr Leu Thr Ala Asp Glu Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser
65 70 75 80

Ser Leu Arg Ser Glu Asp Ser Ala Val Tyr Ser Cys Ala Arg Arg Glu
85 90 95

Thr Thr Thr Val Gly Arg Tyr Tyr Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Thr Val Thr Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
115 120 125

Gly Gly Gly Ser Glu Leu Val Leu Thr Gln Ser Pro Ala Ser Leu Ala
130 135 140

Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser
145 150 155 160

Val Asp Tyr Asp Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro
165 170 175

Gly Gln Pro Pro Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser
180 185 190

Gly Ile Pro Pro Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
195 200 205

Leu Asn Ile His Pro Val Glu Lys Val Asp Ala Ala Thr Tyr His Cys
210 215 220

Gln Gln Ser Thr Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu
225 230 235 240

Glu Ile Lys Arg Arg Ser
245

(22) INFORMATION FOR SEQ ID NO:21

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 241

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: protein

(A) DESCRIPTION: single chain SJ25C1 protein

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: Anti CD-19 hybridomas

(B) CLONE: SJ25C1 cell line

(x) PUBLICATION INFORMATION:

(A) AUTHORS: *

(B) TITLE: *

(C) JOURNAL: *

(D) VOLUME: *

(E) ISSUE: *

(F) PAGES: *

(G) DATE: *

(H) DOCUMENT NUMBER: *

(I) FILING DATE: *

(J) PUBLICATION DATE: *

(K) RELEVANT RESIDUES: *

(xi) A SEQUENCE DESCRIPTION: SEQ ID NO: 21:

Leu Glu Ser Gly Ala Glu Leu Val Arg Pro Gly Ser Ser Val Lys Ile
5 10 15

Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr Trp Met Asn Trp
20 25 30

Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Gln Ile Tyr
35 40 45

Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys Gly Gln Ala
50 55 60

Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser
65 70 75 80

Gly Leu Thr Ser Glu Asp Ser Ala Val Tyr Ser Cys Ala Arg Lys Thr
85 90 95

Ile Ser Ser Val Val Asp Phe Tyr Phe Asp Asn Trp Gly Gln Gly Thr
100 105 110

Thr Val Thr Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
115 120 125

Gly Ser Glu Leu Val Leu Thr Gln Ser Pro Lys Phe Met Ser Thr Ser
130 135 140

Val Gly Asp Arg Val Ser Val Thr Cys Lys Ala Ser Gln Asn Val Gly
145 150 155 160

Thr Asn Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Pro
165 170 175

Leu Ile Tyr Ser Ala Thr Tyr Arg Asn Ser Gly Val Pro Asp Arg Phe
180 185 190

Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Asn Val
195 200 205

Gln Ser Lys Asp Leu Ala Asp Tyr Phe Tyr Phe Cys Gln Tyr Asn Arg
210 215 220

Tyr Pro Tyr Thr Ser Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Arg
225 230 235 240

Ser

(23) INFORMATION FOR SEQ ID NO:22

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 243

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: protein

(A) DESCRIPTION: single chain BLY3 protein

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: Anti CD-19 hybridomas

(B) CLONE: BLY3 cell line

(x) PUBLICATION INFORMATION:

(A) AUTHORS: *

(B) TITLE: *

(C) JOURNAL: *

(D) VOLUME: *

(E) ISSUE: *

(F) PAGES: *

(G) DATE: *

(H) DOCUMENT NUMBER: *

(I) FILING DATE: *

(J) PUBLICATION DATE: *

(K) RELEVANT RESIDUES: *

(xi) A SEQUENCE DESCRIPTION: SEQ ID NO: 22:

Leu Glu Ser Gly Ala Glu Leu Val Arg Pro Gly Ala Ser Val Lys Ile
5 10 15

Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Ser Trp Met Asn Trp
20 25 30

Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Arg Ile Tyr
35 40 45

Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys Glu Ala Ala
50 55 60

Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser
65 70 75 80

Ser Leu Thr Ser Val Asp Ser Ala Val Tyr Ser Cys Ala Arg Ser Glu
85 90 95

Tyr Trp Gly Asn Tyr Trp Ala Met Asp Tyr Trp Gly Gln Gly Thr Thr
100 105 110

Val Thr Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
115 120 125

Ser Glu Leu Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu
130 135 140

Gly Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Gln Ser Val Asp Asn
145 150 155 160

Tyr Gly Ile Ser Phe Met Asn Trp Phe Gln Gln Lys Pro Gly Gln Pro
165 170 175

Pro Lys Leu Leu Ile Tyr Ala Ala Ser Asn Gln Gly Ser Gly Val Pro
180 185 190

Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu Asn Ile
195 200 205

His Pro Met Glu Glu Asp Asp Thr Ala Met Tyr Phe Cys Gln Gln Ser
210 215 220

Lys Glu Val Pro Arg Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
225 230 235 240

Arg Arg Ser

(24) INFORMATION FOR SEQ ID NO:23

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 738

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double stranded

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(A) DESCRIPTION: single chain B43 DNA

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: Anti CD-19 hybridomas

(B) CLONE: B43 cell line

(x) PUBLICATION INFORMATION:

(A) AUTHORS: *

(B) TITLE: *

(C) JOURNAL: *

(D) VOLUME: *

(E) ISSUE: *

(F) PAGES: *

(G) DATE: *

(H) DOCUMENT NUMBER: *

(I) FILING DATE: *

(J) PUBLICATION DATE: *

(K) RELEVANT RESIDUES: *

(xi) A SEQUENCE DESCRIPTION: SEQ ID NO: 23:

```
CTC GAG TCT GGG GCT GAG CTG GTG AGG CCT GGG TCC TCA GTG AAG ATT  48
Leu Glu Ser Gly Ala Glu Leu Val Arg Pro Gly Ser Ser Val Lys Ile
                5                  10                  15

TCC TGC AAG GCT TCT GGC TAT GCA TTC AGT AGC TAC TGG ATG AAC TGG  96
Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr Trp Met Asn Trp
            20                  25                  30

GTG AAG CAG AGG CCT GGA CAG GGT CTT GAG TGG ATT GGA CAG ATT TGG  144
Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Gln Ile Trp
        35                  40                  45

CCT GGA GAT GGT GAT ACT AAC TAC AAT GGA AAG TTC AAG GGT AAA GCC  192
Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys Gly Lys Ala
    50                  55                  60

ACT CTG ACT GCA GAC GAA TCC TCC AGC ACA GCC TAC ATG CAA CTC AGC  240
Thr Leu Thr Ala Asp Glu Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser
65                  70                  75                  80

AGC CTA CGA TCT GAG GAC TCT GCG GTC TAT TCT TGT GCA AGA CGG GAG  288
Ser Leu Arg Ser Glu Asp Ser Ala Val Tyr Ser Cys Ala Arg Arg Glu
                85                  90                  95

ACT ACG ACG GTA GGC CGT TAT TAC TAT GCT ATG GAC TAC TGG GGC CAA  336
Thr Thr Thr Val Gly Arg Tyr Tyr Tyr Ala Met Asp Tyr Trp Gly Gln
            100                 105                 110

GGG ACC ACG GTC ACC GGA GGC GGT GGC TCG GGC GGT GGC GGC TCG GGT  384
Gly Thr Thr Val Thr Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
        115                 120                 125

GGC GGC GGA TCC GAG CTC GTG CTC ACC CAG TCT CCA GCT TCT TTG GCT  432
Gly Gly Gly Ser Glu Leu Val Leu Thr Gln Ser Pro Ala Ser Leu Ala
    130                 135                 140
```

GTG TCT CTA GGG CAG AGG GCC ACC ATC TCC TGC AAG GCC AGC CAA AGT 480
Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser
145 150 155 160

GTT GAT TAT GAT GGT GAT AGT TAT TTG AAC TGG TAC CAA CAG ATT CCA 528
Val Asp Tyr Asp Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro
165 170 175

GGA CAG CCA CCC AAA CTC CTC ATC TAT GAT GCA TCC AAT CTA GTT TCT 576
Gly Gln Pro Pro Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser
180 185 190

GGG ATC CCA CCC AGG TTT AGT GGC AGT GGG TCT GGG ACA GAC TTC ACC 624
Gly Ile Pro Pro Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
195 200 205

CTC AAC ATC CAT CCT GTG GAG AAG GTG GAT GCT GCA ACC TAT CAC TGT 672
Leu Asn Ile His Pro Val Glu Lys Val Asp Ala Ala Thr Tyr His Cys
210 215 220

CAG CAA AGT ACT GAG GAT CCG TGG ACG TTC GGT GGA GGG ACC AAG CTG 720
Gln Gln Ser Thr Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu
225 230 235 240

GAA ATA AAA CGT AGA TCT 738
Glu Ile Lys Arg Arg Ser
245

(25) INFORMATION FOR SEQ ID NO:24

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 741

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double stranded

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(A) DESCRIPTION: single chain SJ25C1 DNA

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: Anti CD-19 hybridomas

(B) CLONE: SJ25C1 cell line

(x) PUBLICATION INFORMATION:

(A) AUTHORS: *

(B) TITLE: *

(C) JOURNAL: *

(D) VOLUME: *

(E) ISSUE: *

(F) PAGES: *

(G) DATE: *

(H) DOCUMENT NUMBER: *

(I) FILING DATE: *

(J) PUBLICATION DATE: *

(K) RELEVANT RESIDUES: *

(xi) A SEQUENCE DESCRIPTION: SEQ ID NO: 24:

```
CTC GAG TCT GGG GCT GAG CTG GTG AGG CCT GGG TCC TCA GTG AAG ATT  48
Leu Glu Ser Gly Ala Glu Leu Val Arg Pro Gly Ser Ser Val Lys Ile
                5                  10                  15

TCC TGC AAG GCT TCT GGC TAT GCA TTC AGT AGC TAC TGG ATG AAC TGG  96
Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr Trp Met Asn Trp
            20                  25                  30

GTG AAG CAG AGG CCT GGA CAG GGT CTT GAG TGG ATT GGA CAG ATT TAT 144
Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Gln Ile Tyr
        35                  40                  45

CCT GGA GAT GGT GAT ACT AAC TAC AAT GGA AAG TTC AAG GGT CAA GCC 192
Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys Gly Gln Ala
    50                  55                  60

ACA CTG ACT GCA GAC AAA TCC TCC AGC ACA GCC TAC ATG CAG CTC AGC 240
Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser
65                  70                  75                  80

GGC CTA ACA TCT GAG GAC TCT GCG GTC TAT TCT TGT GCA AGA AAG ACC 288
Gly Leu Thr Ser Glu Asp Ser Ala Val Tyr Ser Cys Ala Arg Lys Thr
                85                  90                  95

ATT AGT TCG GTA GTA GAT TTC TAC TTT GAC AAC TGG GGC CAA GGG ACC 336
Ile Ser Ser Val Val Asp Phe Tyr Phe Asp Asn Trp Gly Gln Gly Thr
            100                 105                 110

ACG GTC ACC GGA GGC GGT GGC TCG GGC GGT GGC GGC TCG GGT GGC GGC 384
Thr Val Thr Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
        115                 120                 125

GGA TCC GAG CTC GTG CTC ACC CAG TCT CCA AAA TTC ATG TCC ACA TCA 432
Gly Ser Glu Leu Val Leu Thr Gln Ser Pro Lys Phe Met Ser Thr Ser
    130                 135                 140
```

```
GTA GGA GAC AGG GTC AGC GTC ACC TGC AAG GCC AGT CAG AAT GTG GGT  528
Val Gly Asp Arg Val Ser Val Thr Cys Lys Ala Ser Gln Asn Val Gly
145                 150                 155                 160

ACT AAT GTA GCC TGG TAT CAA CAG AAA CCA GGA CAA TCT CCT AAA CCA  576
Thr Asn Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Pro
                165                 170                 175

CTG ATT TAC TCG GCA ACC TAC CGG AAC AGT GGA GTC CCT GAC CGC TTC  624
Leu Ile Tyr Ser Ala Thr Tyr Arg Asn Ser Gly Val Pro Asp Arg Phe
            180                 185                 190

ACA GGC AGT GGA TCT GGG ACA GAT TTC ACT CTC ACC ATC ACT AAC GTG  672
Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Asn Val
        195                 200                 205

CAG TCT AAA GAC TTG GCA GAC TAT TTC TAT TTC TGT CAA TAT AAC AGG  720
Gln Ser Lys Asp Leu Ala Asp Tyr Phe Tyr Phe Cys Gln Tyr Asn Arg
    210                 215                 220

TAT CCG TAC ACG TCC GGA GGG GGG ACC AAG CTG GAA ATA AAA CGT AGA  738
Tyr Pro Tyr Thr Ser Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Arg
225                 230                 235                 240

TCT                                                             741
Ser
```

(26) INFORMATION FOR SEQ ID NO:25

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 729

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double stranded

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(A) DESCRIPTION: single chain BLY3 DNA

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: Anti CD-19 hybridomas

(B) CLONE: BLY3 cell line

(x) PUBLICATION INFORMATION:

(A) AUTHORS: *

(B) TITLE: *

(C) JOURNAL: *

(D) VOLUME: *

(E) ISSUE: *

(F) PAGES: *

(G) DATE: *

(H) DOCUMENT NUMBER: *

(I) FILING DATE: *

(J) PUBLICATION DATE: *

(K) RELEVANT RESIDUES: *

(xi) A SEQUENCE DESCRIPTION: SEQ ID NO: 25:

```
CTC GAG TCT GGG GCT GAG CTG GTG AGG CCT GGG GCC TCA GTG AAG ATT  48
Leu Glu Ser Gly Ala Glu Leu Val Arg Pro Gly Ala Ser Val Lys Ile
                5                  10                  15

TCC TGC AAA GCT TCT GGC TAC GCA TTC AGT AGC TCT TGG ATG AAC TGG  96
Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Ser Trp Met Asn Trp
            20                  25                  30

GTG AAG CAG AGG CCT GGA CAG GGT CTT GAG TGG ATT GGA CGG ATT TAT 144
Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Arg Ile Tyr
        35                  40                  45

CCT GGA GAT GGA GAT ACT AAC TAC AAT GGA AAG TTC AAG GAA GCG GCC 192
Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys Glu Ala Ala
    50                  55                  60

ACA CTG ACT GCA GAC AAA TCC TCC AGC ACA GCG TAC ATG CAG CTC AGC 240
Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser
65                  70                  75                  80

AGC CTG ACC TCT GTG GAC TCT GCG GTC TAT TCT TGT GCA AGA TCG GAG 288
Ser Leu Thr Ser Val Asp Ser Ala Val Tyr Ser Cys Ala Arg Ser Glu
                85                  90                  95

TAT TGG GGT AAC TAC TGG GCT ATG GAC TAC TGG GGC CAA GGG ACC ACG 336
Tyr Trp Gly Asn Tyr Trp Ala Met Asp Tyr Trp Gly Gln Gly Thr Thr
            100                 105                 110

GTC ACC GGA GGC GGT GGC TCG GGC GGT GGC GGC TCG GGT GGC GGC GGA 384
Val Thr Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
        115                 120                 125
```

```
TCC GAG CTC GTG CTC ACC CAG TCT CCA GCT TCT TTG GCT GTG TCT CTA  432
Ser Glu Leu Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu
    130                 135                 140

GGG CAG AGG GCC ACC ATC TCC TGC AGA GCC AGC CAG AGT GTT GAT AAT  480
Gly Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Gln Ser Val Asp Asn
145                 150                 155                 160

TAT GGC ATT AGT TTT ATG AAC TGG TTC CAA CAG AAA CCA GGA CAG CCA  528
Tyr Gly Ile Ser Phe Met Asn Trp Phe Gln Gln Lys Pro Gly Gln Pro
                165                 170                 175

CCC AAA CTC CTC ATC TAT GCT GCA TCC AAC CAA GGA TCC GGG GTC CCT  576
Pro Lys Leu Leu Ile Tyr Ala Ala Ser Asn Gln Gly Ser Gly Val Pro
            180                 185                 190

GCC AGG TTT AGT GGC AGT GGG TCT GGG ACA GAC TTC AGC CTC AAC ATC  624
Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu Asn Ile
        195                 200                 205

CAT CCT ATG GAG GAG GAT GAT ACT GCA ATG TAT TTC TGT CAG CAA AGT  672
His Pro Met Glu Glu Asp Asp Thr Ala Met Tyr Phe Cys Gln Gln Ser
    210                 215                 220

AAG GAG GTT CCT CGG ACG TTC GGT GGA GGG ACC AAG CTG GAA ATA AAA  720
Lys Glu Val Pro Arg Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
225                 230                 235                 240

CGT AGA TCT                                                     729
Arg Arg Ser
```

(27) INFORMATION FOR SEQ ID NO:26

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 247

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: protein

(A) DESCRIPTION: modified single chain B43 antibody

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: Anti CD-19 hybridomas

(B) CLONE: B43 cell line

(x) PUBLICATION INFORMATION:

(A) AUTHORS: *

(B) TITLE: *

(C) JOURNAL: *

(D) VOLUME: *

(E) ISSUE: *

(F) PAGES: *

(G) DATE: *

(H) DOCUMENT NUMBER: *

(I) FILING DATE: *

(J) PUBLICATION DATE: *

(K) RELEVANT RESIDUES: *

(xi) A SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Leu Glu Ser Gly Ala Glu Leu Val Arg Pro Gly Ser Ser Val Lys Ile
5 10 15

Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr Trp Met Asn Trp
20 25 30

Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Gln Ile Trp
35 40 45

Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys Gly Lys Ala
50 55 60

Thr Leu Thr Ala Asp Glu Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser
65 70 75 80

Ser Leu Arg Ser Glu Asp Ser Ala Val Tyr Ser Cys Ala Arg Arg Glu
85 90 95

Thr Thr Thr Val Gly Arg Tyr Tyr Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Thr Val Thr Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
115 120 125

Gly Gly Gly Ser Glu Leu Val Leu Thr Gln Ser Pro Ala Ser Leu Ala
130 135 140

Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser
145 150 155 160

Val Asp Tyr Asp Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro
165 170 175

Gly Gln Pro Pro Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser
180 185 190

Gly Ile Pro Pro Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
195 200 205

Leu Asn Ile His Pro Val Glu Lys Val Asp Ala Ala Thr Tyr His Cys
210 215 220

Gln Gln Ser Thr Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu
225 230 235 240

Glu Ile Lys Arg Arg Ser Cys
245

(28) INFORMATION FOR SEQ ID NO:27

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 242

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: protein

(A) DESCRIPTION: modified single chain SJ25C1 antibody

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: Anti CD-19 hybridomas

(B) CLONE: SJ25C1 cell line

(x) PUBLICATION INFORMATION:

(A) AUTHORS: *

(B) TITLE: *

(C) JOURNAL: *

(D) VOLUME: *

(E) ISSUE: *

(F) PAGES: *

(G) DATE: *

(H) DOCUMENT NUMBER: *

(I) FILING DATE: *

(J) PUBLICATION DATE: *

(K) RELEVANT RESIDUES: *

(xi) A SEQUENCE DESCRIPTION: SEQ ID NO: 27:

Leu Glu Ser Gly Ala Glu Leu Val Arg Pro Gly Ser Ser Val Lys Ile
5 10 15

Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr Trp Met Asn Trp
20 25 30

Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Gln Ile Tyr
35 40 45

Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys Gly Gln Ala
50 55 60

Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser
65 70 75 80

Gly Leu Thr Ser Glu Asp Ser Ala Val Tyr Ser Cys Ala Arg Lys Thr
85 90 95

Ile Ser Ser Val Val Asp Phe Tyr Phe Asp Asn Trp Gly Gln Gly Thr
100 105 110

Thr Val Thr Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
115 120 125

Gly Ser Glu Leu Val Leu Thr Gln Ser Pro Lys Phe Met Ser Thr Ser
130 135 140

Val Gly Asp Arg Val Ser Val Thr Cys Lys Ala Ser Gln Asn Val Gly
145 150 155 160

Thr Asn Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Pro
165 170 175

Leu Ile Tyr Ser Ala Thr Tyr Arg Asn Ser Gly Val Pro Asp Arg Phe
180 185 190

Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Asn Val
195 200 205

Gln Ser Lys Asp Leu Ala Asp Tyr Phe Tyr Phe Cys Gln Tyr Asn Arg
210 215 220

Tyr Pro Tyr Thr Ser Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Arg
225 230 235 240

Ser Cys

(29) INFORMATION FOR SEQ ID NO:28

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 494

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: protein

(A) DESCRIPTION: dimer of single chain B43 antibody

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: Anti CD-19 hybridomas

(B) CLONE: B43 cell line

(x) PUBLICATION INFORMATION:

(A) AUTHORS: *

(B) TITLE: *

(C) JOURNAL: *

(D) VOLUME: *

(E) ISSUE: *

(F) PAGES: *

(G) DATE: *

(H) DOCUMENT NUMBER: *

(I) FILING DATE: *

(J) PUBLICATION DATE: *

(K) RELEVANT RESIDUES: *

(xi) A SEQUENCE DESCRIPTION: SEQ ID NO: 28:

Leu Glu Ser Gly Ala Glu Leu Val Arg Pro Gly Ser Ser Val Lys Ile
5 10 15

Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr Trp Met Asn Trp
20 25 30

Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Gln Ile Trp
35 40 45

Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys Gly Lys Ala
50 55 60

Thr Leu Thr Ala Asp Glu Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser
65 70 75 80

Ser Leu Arg Ser Glu Asp Ser Ala Val Tyr Ser Cys Ala Arg Arg Glu
85 90 95

Thr Thr Thr Val Gly Arg Tyr Tyr Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Thr Val Thr Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
115 120 125

Gly Gly Gly Ser Glu Leu Val Leu Thr Gln Ser Pro Ala Ser Leu Ala
130 135 140

Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser
145 150 155 160

Val Asp Tyr Asp Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro
165 170 175

Gly Gln Pro Pro Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser
180 185 190

Gly Ile Pro Pro Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
195 200 205

Leu Asn Ile His Pro Val Glu Lys Val Asp Ala Ala Thr Tyr His Cys
210 215 220

Gln Gln Ser Thr Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu
225 230 235 240

Glu Ile Lys Arg Arg Ser Cys Cys Ser Arg Arg Lys Ile Glu Leu Lys
245 250 255

Thr Gly Gly Gly Phe Thr Trp Pro Asp Glu Thr Ser Gln Gln Cys His
260 265 270

Tyr Thr Ala Ala Asp Val Lys Glu Val Pro His Ile Asn Leu Thr Phe
275 280 285

Asp Thr Gly Ser Gly Ser Gly Ser Phe Arg Pro Pro Ile Gly Ser Val
290 295 300

Leu Asn Ser Ala Asp Tyr Ile Leu Leu Lys Pro Pro Gln Gly Pro Ile
305 310 315 320

Gln Gln Tyr Trp Asn Leu Tyr Ser Asp Gly Asp Tyr Asp Val Ser Gln
325 330 335

Ser Ala Lys Cys Ser Ile Thr Ala Arg Gln Gly Leu Ser Val Ala Leu
340 345 350

Ser Ala Pro Ser Gln Thr Leu Val Leu Glu Ser Gly Gly Gly Gly Ser

355 360 365

Gly Gly Gly Gly Ser Gly Gly Gly Gly Thr Val Thr Thr Gly Gln Gly
370 375 380

Trp Tyr Asp Met Ala Tyr Tyr Tyr Arg Gly Val Thr Thr Thr Glu Arg
385 390 395 400

Arg Ala Cys Ser Tyr Val Ala Ser Asp Glu Ser Arg Leu Ser Ser Leu
405 410 415

Gln Met Tyr Ala Thr Ser Ser Ser Glu Asp Ala Thr Leu Thr Ala Lys
420 425 430

Gly Lys Phe Lys Gly Asn Tyr Asn Thr Asp Gly Asp Gly Pro Trp Ile
435 440 445

Gln Gly Ile Trp Glu Leu Gly Gln Gly Pro Arg Gln Lys Val Trp Asn
450 455 460

Met Trp Tyr Ser Ser Phe Ala Tyr Gly Ser Ala Lys Cys Ser Ile Lys
465 470 475 480

Val Ser Ser Gly Pro Arg Val Leu Glu Ala Gly Ser Glu Leu
485 490

(30) INFORMATION FOR SEQ ID NO:29

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 484

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: protein

(A) DESCRIPTION: dimer of single chain SJ25C1 antibody

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: Anti CD-19 hybridomas

(B) CLONE: SJ25C1 cell line

(x) PUBLICATION INFORMATION:

(A) AUTHORS: *

(B) TITLE: *

(C) JOURNAL: *

(D) VOLUME: *

(E) ISSUE: *

(F) PAGES: *

(G) DATE: *

(H) DOCUMENT NUMBER: *

(I) FILING DATE: *

(J) PUBLICATION DATE: *

(K) RELEVANT RESIDUES: *

(xi) A SEQUENCE DESCRIPTION: SEQ ID NO: 29:

Leu Glu Ser Gly Ala Glu Leu Val Arg Pro Gly Ser Ser Val Lys Ile
5 10 15

Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr Trp Met Asn Trp
20 25 30

Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Gln Ile Tyr
35 40 45

Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys Gly Gln Ala
50 55 60

Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser
65 70 75 80

Gly Leu Thr Ser Glu Asp Ser Ala Val Tyr Ser Cys Ala Arg Lys Thr
85 90 95

Ile Ser Ser Val Val Asp Phe Tyr Phe Asp Asn Trp Gly Gln Gly Thr
100 105 110

Thr Val Thr Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
115 120 125

Gly Ser Glu Leu Val Leu Thr Gln Ser Pro Lys Phe Met Ser Thr Ser
130 135 140

Val Gly Asp Arg Val Ser Val Thr Cys Lys Ala Ser Gln Asn Val Gly
145 150 155 160

Thr Asn Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Pro
165 170 175

Leu Ile Tyr Ser Ala Thr Tyr Arg Asn Ser Gly Val Pro Asp Arg Phe
180 185 190

Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Asn Val
195 200 205

Gln Ser Lys Asp Leu Ala Asp Tyr Phe Tyr Phe Cys Gln Tyr Asn Arg
210 215 220

Tyr Pro Tyr Thr Ser Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Arg
225 230 235 240

Ser Cys Cys Ser Arg Arg Lys Ile Glu Leu Lys Thr Gly Gly Gly Ser
245 250 255

Thr Tyr Pro Tyr Arg Asn Tyr Gln Cys Phe Tyr Phe Tyr Asp Ala Leu
260 265 270

Asp Lys Ser Gln Val Asn Thr Ile Thr Leu Thr Phe Asp Thr Gly Ser
275 280 285

Gly Ser Gly Thr Phe Arg Asp Pro Val Gly Ser Asn Arg Tyr Thr Ala
290 295 300

Ser Tyr Ile Leu Pro Lys Pro Ser Gln Gly Pro Lys Gln Gln Tyr Trp
305 310 315 320

Ala Val Asn Thr Gly Val Asn Gln Ser Ala Lys Cys Thr Val Ser Val
325 330 335

Arg Asp Gly Val Ser Thr Ser Met Phe Lys Pro Ser Gln Thr Leu Val
340 345 350

Leu Glu Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
355 360 365

Gly Thr Val Thr Thr Gly Gln Gly Trp Asn Asp Phe Tyr Phe Asp Val
370 375 380

Val Ser Ser Ile Thr Lys Arg Ala Cys Ser Tyr Val Ala Ser Asp Glu
385 390 395 400

Ser Thr Leu Gly Ser Leu Gln Met Tyr Ala Thr Ser Ser Ser Lys Asp

405 410 415

Ala Thr Leu Thr Ala Gln Gly Lys Phe Lys Gly Asn Tyr Asn Thr Asp
420 425 430

Gly Asp Gly Pro Tyr Ile Gln Gly Ile Trp Glu Leu Gly Gln Gly Pro
435 440 445

Arg Gln Lys Val Trp Asn Met Trp Tyr Ser Ser Phe Ala Tyr Gly Ser
450 455 460

Ala Lys Cys Ser Ile Lys Val Ser Ser Gly Pro Arg Val Leu Glu Ala
465 470 475 480

Gly Ser Glu Leu

:19 Protein sequence of BLY3 Light chain

What is claimed is:

1. An isolated and purified polynucleotide encoding a single chain variable region polypeptide that binds to a CD19 antigen.

2. The isolated and purified polynucleotide of claim 1, wherein the polypeptide encoded comprises an amino acid residue sequence according to SEQ ID NO: 20, 21 or 22.

3. The isolated and purified polynucleotide of claim 1 wherein the polynucleotide comprises a nucleotide sequence according to SEQ ID NO: 23, 24 or 25.

4. An isolated and purified polynucleotide comprising a nucleotide base sequence that is identical or complimentary to a segment of at least 10 contiguous nucleotide bases of SEQ ID NO: 23, 24 or 25, wherein the polynucleotide hybridizes to a polynucleotide that encodes a single chain variable region polypeptide that binds to a CD19 antigen.

5. The isolated and purified polynucleotide of claim 4, wherein the encoded polypeptide binds to a CD19 antigen with a K_a of at least 1×10^9 M^1.

6. An isolated and purified polynucleotide comprising a nucleotide base sequence that is identical or complimentary to a segment of at least 100 contiguous nucleotide bases of SEQ ID NO: 23, 24 or 25, wherein the polynucleotide hybridizes to a polynucleotide that encodes a single chain variable region polypeptide that binds to a CD19 antigen.

7. An isolated and purified single chain variable region polypeptide that binds to a CD19 antigen.

8. The isolated and purified polypeptide of claim 7, wherein the polypeptide has a molecular weight of approximately 28 kDa.

9. The isolated and purified polypeptide of claim 7, wherein the polypeptide binds to a CD19 antigen with a K_a of at least 1×10^9 M^{-1}.

10. The isolated and purified polypeptide of claim 7, wherein the polypeptide comprises an amino acid residue sequence according to SEQ ID NO: 20, 21 or 22.

11. The isolated and purified polypeptide of claim 7, wherein the polypeptide is further modified by the site specific insertion of a cysteine residue at the C-terminus of the polypeptide.

12. A dimer of an isolated and purified single chain variable region polypeptide, wherein the dimer is prepared by linking a first polypeptide of claim 11 with a second polypeptide of claim 11 through a disulfide bond between a C-terminus cysteine residue on each polypeptide.

13. An isolated and purified single chain variable region polypeptide that binds to a CD19 antigen, wherein the polypeptide is prepared by a process comprising the steps of:

(A.) cloning a DNA sequence that encodes the polypeptide into an expression vector;

(B.) transforming *E. Coli* cells with the expression vector; and

(C.) expressing the polypeptide in the transformed cells.

14. An immunoconjugate for the treatment of cancer comprising a single chain variable region polypeptide that binds to a CD19 antigen, wherein the polypeptide is linked to at least one cytotoxic agent.

15. The immunoconjugate of claim 14, wherein the polypeptide comprises an amino acid residue sequence according to SEQ ID NO: 20, 21 or 22.

16. The immunoconjugate of claim 14, wherein the at least one cytotoxic agent is selected from the group consisting of single chain, double chain, and multiple chain toxins.

17. The immunoconjugate of claim 14, wherein the at least one cytotoxic agent is a radionuclide selected from the group consisting of beta-emitting metallic radionuclides, alpha emitters, and gamma emitters.

18. An immunoconjugate for the treatment of cancer comprising a polypeptide of claim 12, wherein the polypeptide is linked to at least one cytotoxic agent.

19. The immunoconjugate of claim 18, wherein the at least one cytotoxic agent is selected from the group consisting of single chain, double chain, and multiple chain toxins.

20. The immunoconjugate of claim 18, wherein the at least one cytotoxic agent is a radionuclide selected from the group consisting of beta-emitting metallic radionuclides, alpha emitters, and gamma emitters.

21. A process for preparing an immunoconjugate comprising a single chain variable region polypeptide that binds to a CD19 antigen, wherein the process comprises the steps of:

(D.) preparing the polypeptide according to a method comprising the steps of:

i) cloning a DNA sequence that encodes the polypeptide into an expression vector;

ii) transforming *E. coli* cells with the expression

vector; and

iii) maintaining the transformed cells under biological conditions sufficient for expression of the polypeptide.

(E.) providing a suitable toxin; and

(F.) conjugating the polypeptide to the toxin.

22. The process of claim 21, wherein the process further comprises the step of labelling the immunoconjugate with a radionuclide.

23. An immunoconjugate for the treatment of cancer comprising a polypeptide of claim 12, wherein the polypeptide is linked to at least one cytotoxic agent.

24. The immunoconjugate of claim 23, wherein the at least one cytotoxic agent is selected from the group consisting of single chain, double chain, and multiple chain toxins.

25. The immunoconjugate of claim 23, wherein the at least one cytotoxic agent is a radionuclide selected from the group consisting of beta-emitting metallic radionuclides, alpha emitters, and gamma emitters.

26. An immunoconjugate for the treatment of cancer comprising a polypeptide of claim 12, wherein the polypeptide is linked to at least one cytotoxic agent.

27. The immunoconjugate of claim 18, wherein the at least one cytotoxic agent is selected from the group consisting of single chain, double chain, and multiple chain toxins.

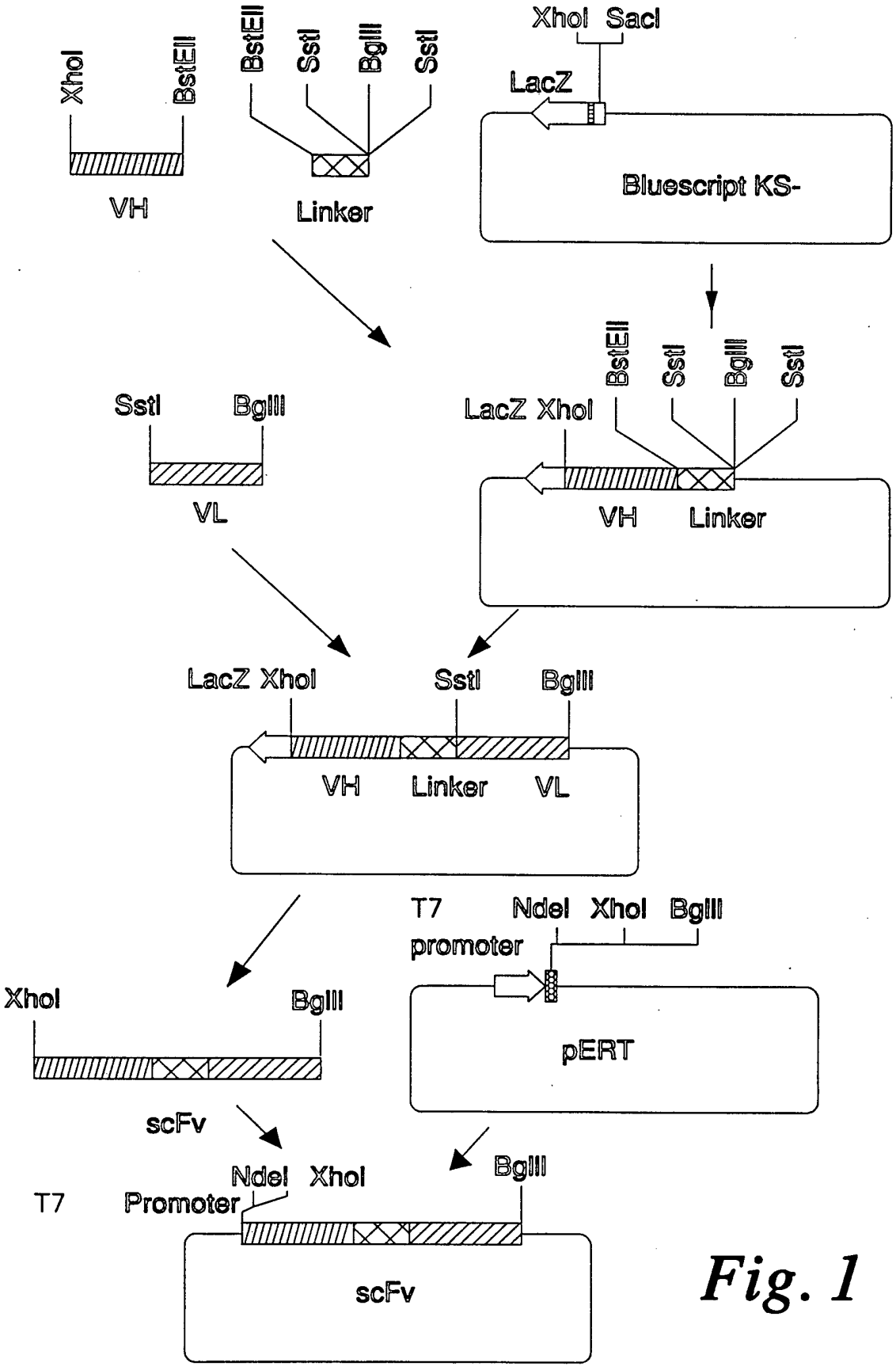
28. The immunoconjugate of claim 18, wherein the at least one cytotoxic agent is a radionuclide selected from the group consisting of beta-

emitting metallic radionuclides, alpha emitters, and gamma emitters.

29. A method for the treatment of cancer comprising the steps of:

(G.) selecting a patient evidencing symptoms of a B-cell cancer, wherein the cancer is selected from the group consisting of leukemia and B-cell lymphoma;

(H.) administering to the patient a therapeutically effective amount of the immunoconjugate of claim 22 in a biocompatible dosage form.


XhoI
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VH
BstEII
SstI
BglII
SstI
Linker
XhoI SacI
LacZ
Bluescript KS-
SstI
BglII
VL
BstEII
SstI
BglII
SstI
LacZ XhoI
VH
Linker
LacZ XhoI
SstI
BglII
VH
Linker
VL
T7
promoter
NdeI
XhoI
BglII
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T7
Promoter
BglII
scFv

Fig. 1

Fig. 2

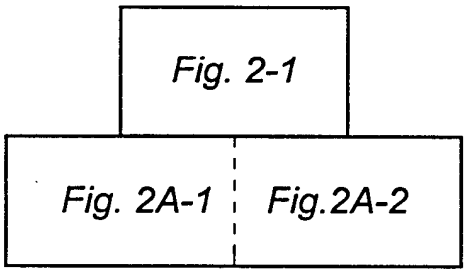


Fig. 3

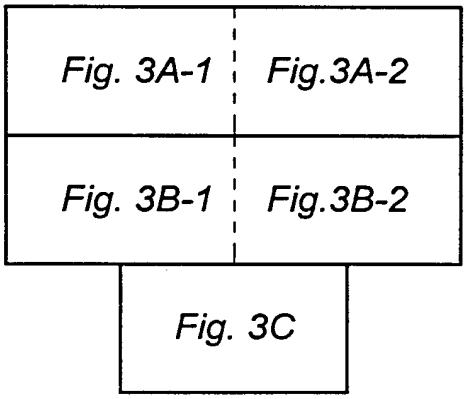


Fig. 2-1

Heavy chain regions

	FR1	CDR2	FR2	CDR2
B43	**LESG**AELVRPGSSVKISCKASGYAFS	SYWMN	WVKQRPGQGLEWIG	QIWPGDGDTNYNGKFKG
25C1	-------------------------	-----	----------	--Y----------
BLY3	----------A----------	-S---	----------	R-Y----------E

	FR3	CDR3
B43	KATLTADESSSTAYMQLSSLRSEDSAVYSCAR	RETTTVGRYYYAMDY**WGQGTTVT**
25C1	Q------K----------G-T----------	KTISS-VDF-F-- --------
BLY3	A------K----------T-V---------	S-YW GN W----------

Light chain regions

	FR1	CDR1	FR2	CDR2
B43	**ELVLTQSP**ASLAVSLGQRATISC	KASQSVDYDGDSYLN	WYQQIPGQPPKLLIY	DASNLVS
25C1	--------KFMST-V-G-V-VT-	----N-GTNVA	----K---S--P---	S-TYRN-
BLY3	----------	R------NY-I-FM-	-F--K----------	A---QG-

	FR3	CDR3
B43	GIPPRFSGSGSGTDFTLNIHPVEKVDAATYHC	QQSTED PWTFGG**GTKLEIKRRS**
25C1	-V-D--T----------T-TN-QSK-L-D-FY	FCQYNRY-Y-S----------
BLY3	-V-A----------S-----M-ED-T-M-FC	---K-V -R ----------

Fig. 2A-1

A

```
     FR1
CDR1
B43  CTC GAG TCT GGG GCT GAG CTG GTG AGG CCT GGG TCC TCA GTG AAG ATT TCC TGC AAG GCT
TCT GGC TAT GCA TTC AGT AGC TAC TGG ATG
25C1 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -
-- --- --- --- --- --- --- --- ---
BLY3 --- --- --- --- --- --- --- --- --- --- --- G-- --- --- --- --- --- --- --A --- --- -
-- --C --- --- --- --- -CT --- ---


          FR2                                                              CDR2
B43  AAC TGG GTG AAG CAG AGG CCT GGA CAG GGT CTT GAG TGG ATT GGA CAG ATT TGG CCT GGA GAT
GGT GAT ACT AAC TAC AAT GGA AAG TTC
25C1 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -AT --- --- --- -
-- --- --- --- --- --- --- --- ---
BLY3 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- CGG --- -AT --- --- --- -
-A --- --- --- --- --- --- --- ---

     CDR2     FR3
B43  AAG GGT AAA GCC ACT CTG ACT GCA GAC GAA TCC TCC AGC ACA GCC TAC ATG CAA CTC AGC AGC
CTA CGA TCT GAG GAC TCT GCG GTC TAT
25C1 --- --- C-- --- --A --- --- --- --- A-- --- --- --- --- --- --- --- --G --- --- G-- -
-- AC- --- --- --- --- --- --- ---
BLY3 --- -AA GCG --- --A --- --- --- --- A-- --- --- --- --- --G --- --- --G --- --- --- -
-G ACC --- -T- --- --- --- --- ---

      FR3              CDR3
B43   TCT TGT GCA AGA CGG GAG ACT ACG ACG GTA GGC CGT TAT TAC TAT GCT ATG GAC TAC TGG
GGC CAA GGG ACC ACG GTC ACC
25C1  --- --- --- --- AA- ACC -T- -GT T-- --- -TA GA- -TC --- -T- -AC -AC          --- ---
--- --- --- --- --- ---
BLY3  --- --- --- --- TC- --- TA- TG-     -GT AA-          --- -GG --- --- --- --- --- ---
--- --- --- --- --- ---
```

Fig. 2A-2

B

FR1

CDR1

B43 **GAG CTC GTG CTC ACC CAG TCT CCA** GCT TCT TTG GCT GTG TCT CTA GGG CAG AGG GCC ACC ATC TCC TGC AAG GCC AGC CAA AGT GTT GAT

25C1 --- --- --- --- --- --- --- --- AAA -TC A-- T-C ACA --A G-- --A G-C --- -T- -G- G-- A-- --- --- --- --T --G -A- --G -G-

BLY3 --- -- --- -GA --- --- --G --- --- ---

FR2

CDR2

B43 TAT GAT GGT GAT AGT TAT TTG AAC TGG TAC CAA CAG ATT CCA GGA CAG CCA CCC AAA CTC CTC ATC TAT GAT GCA TCC AAT CTA GTT TCT

25C1 AC- A-- -TA -CC --- --T --- --- -AA --- --- --A T-T --T --- -CA --G --T --C TCG --- A-- T-C -GG AAC AG-

BLY3 A-- T-- --C AT- --- TTT A-- --- --- -T- --- --- -AA --- --- --- --- --- --- --- --- -- --- -C- --- --- --C -A- -GA --C

FR3

B43 GGG ATC CCA CCC AGG TTT AGT GGC AGT GGG TCT GGG ACA GAC TTC ACC CTC AAC ATC CAT CCT GTG GAG AAG GTG GAT GCT GCA ACC TAT

25C1 --A G-- --T GA- C-C --C -CA --- --- --A --- --- --- --T --- --T --- -CC --- AC- AAC -- C-- TCT AAA --C TTG --- GA- ---

BLY3 --- G-- --T G-- --- --- --- --- --- --- --- --- --- --- --- -G- --- --- --- --- --- A-- --- G-- -AT --- A-- --- -TG ---

CDR3

B43 CAC TGT CAG CAA AGT ACT GAG GAT CCG TGG ACG TTC GGT GGA GGG **ACC AAG CTG GAA ATA AAA CGT AGA TCT**

24C1 TT- -A- TTC TGT CAA TA- A-C AGG TAT --- -AC --- -C- --A --G --- --- --- --- --- --- -- --- --- ---

BLY3 TT- --- --- --- --- -AG --- -T- --T C-- --- --- --- --- --- --- --- --- --- --- --- -- --- --- ---

Fig. 3A-1

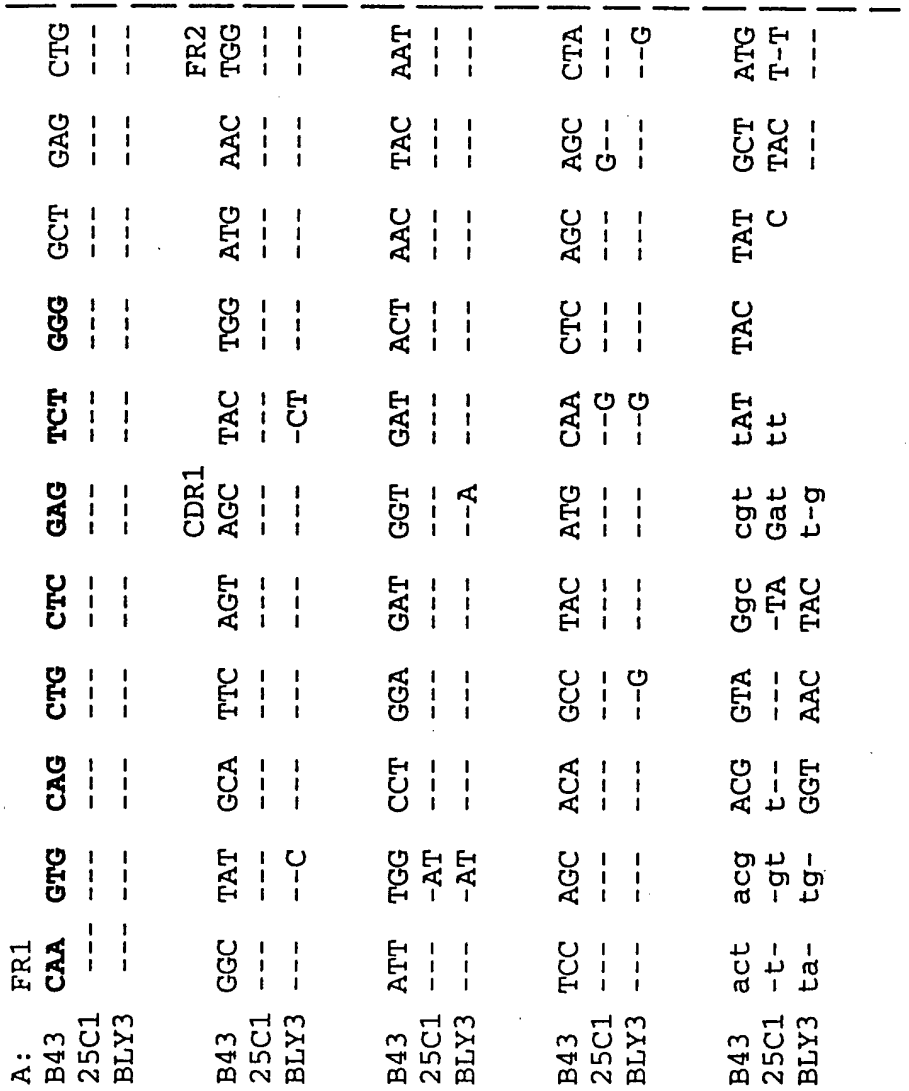

A: FR1
B43 CAA GTG CAG CTG CTC GAG TCT GGG GCT GAG CTG
25C1 --- --- --- --- --- --- --- --- --- ---
BLY3 --- --- --- --- --- --- --- --- --- ---
CDR1 FR2
B43 GGC TAT GCA TTC AGT AGC TAC TGG ATG AAC TGG
25C1 --- --- --- --- --- --- --- --- --- ---
BLY3 --- --C --- --- --- -CT --- --- --- ---
B43 ATT TGG CCT GGA GAT GGT GAT ACT AAC TAC AAT
25C1 --- -AT --- --- --- --- --- --- --- ---
BLY3 --- -AT --- --- --- --A --- --- --- ---
B43 TCC AGC ACA GCC TAC ATG CAA CTC AGC AGC CTA
25C1 --- --- --- --- --- --- --G --- --- ---
BLY3 --- --- --- --G --- --- --G --- --- --G
B43 act acg ACG GTA Ggc cgt tAT TAC TAT GCT ATG
25C1 -t- -gt t-- --- -TA Gat tt C TAC T-T
BLY3 ta- tg- GGT AAC TAC t-g --- ---

Fig. 3A-2

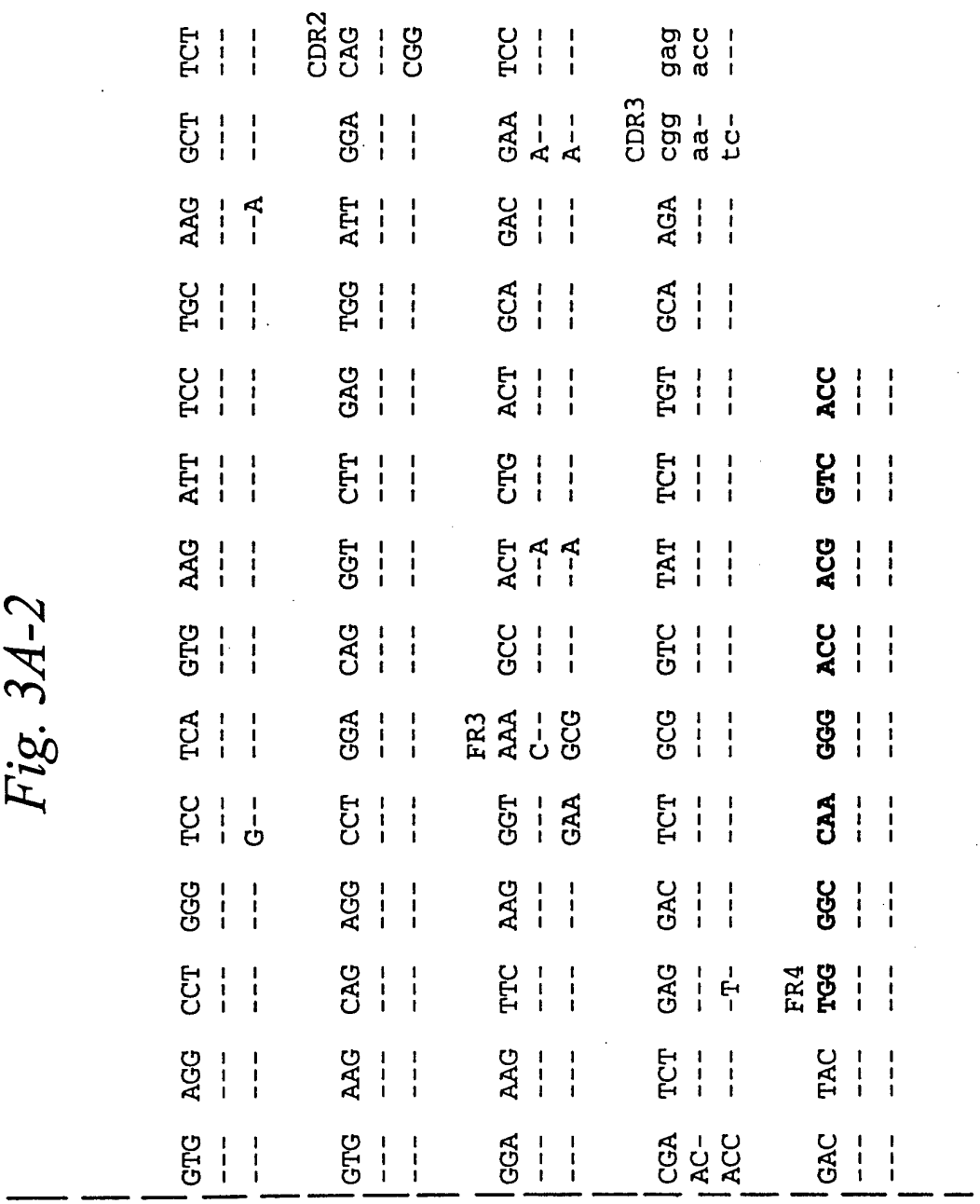

GTG AGG CCT GGG TCC TCA GTG AAG ATT TCC TGC AAG GCT TCT
--- --- --- --- --- --- --- --- --- --- --- --- --- ---
--- --- --- --- G-- --- --- --- --- --- --- --A --- ---
CDR2
GTG AAG CAG AGG CCT GGA CAG GGT CTT GAG TGG ATT GGA CAG
--- --- --- --- --- --- --- --- --- --- --- --- --- ---
--- --- --- --- --- --- --- --- --- --- --- --- --- CGG
FR3
GGA AAG TTC AAG GGT AAA GCC ACT CTG ACT GCA GAC GAA TCC
--- --- --- --- --- C-- --- --A --- --- --- --- A-- ---
--- --- --- --- GAA GCG --- --A --- --- --- --- A-- ---
CDR3
CGA TCT GAG GAC TCT GCG GTC TAT TCT TGT GCA AGA cgg gag
AC- --- --- --- --- --- --- --- --- --- --- --- aa- acc
ACC --- -T- --- --- --- --- --- --- --- --- --- tc- ---
FR4
GAC TAC TGG GGC CAA GGG ACC ACG GTC ACC
--- --- --- --- --- --- --- --- --- --- --- --- --- ---
--- --- --- --- --- --- --- --- --- --- --- --- --- ---

Fig. 3B-1

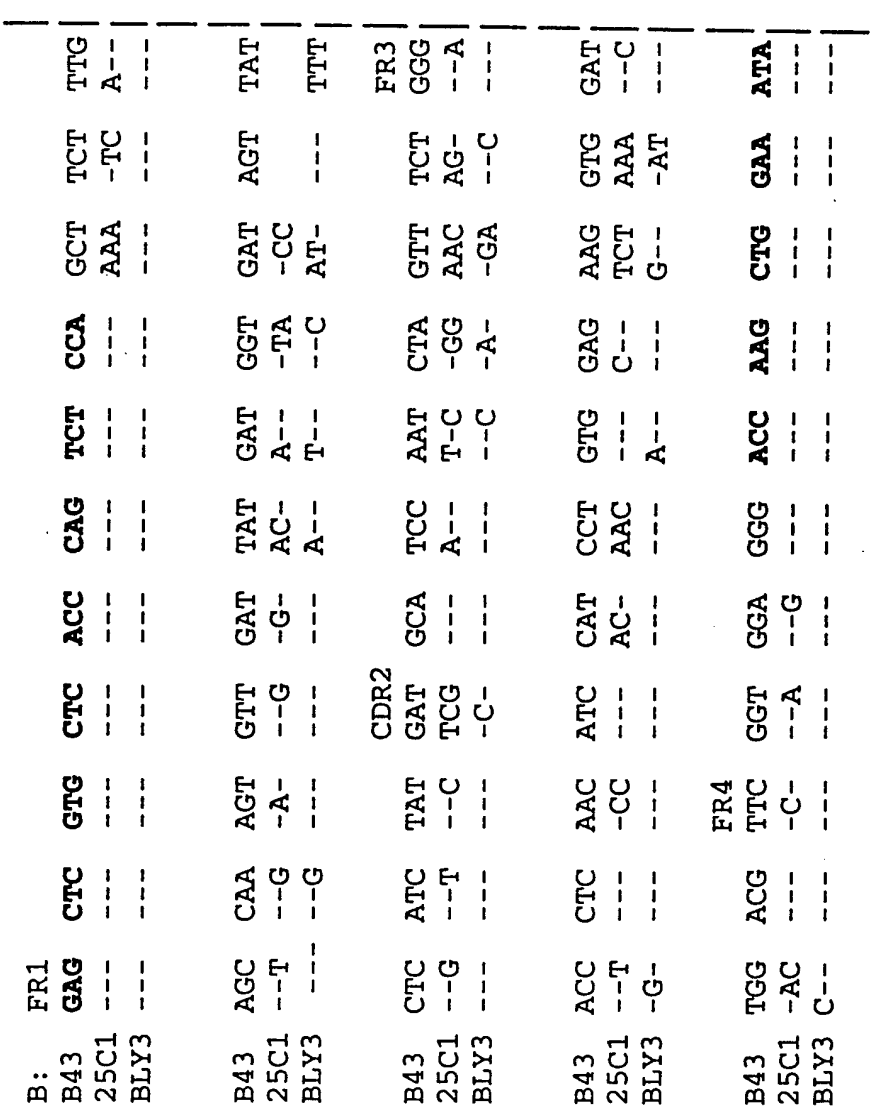

B: FR1
B43 GAG CTC GTG CTC ACC CAG TCT CCA GCT TCT TTG
25C1 --- --- --- --- --- --- --- --- AAA -TC A--
BLY3 --- --- --- --- --- --- --- --- --- --- ---
B43 AGC CAA AGT GTT GAT TAT GAT GGT GAT AGT TAT
25C1 --T --G -A- --G -G- AC- A-- -TA -CC
BLY3 --- --G --- --- --- A-- T-- --C AT- --- TTT
CDR2 FR3
B43 CTC ATC TAT GAT GCA TCC AAT CTA GTT TCT GGG
25C1 --G --T --C TCG --- A-- T-C -GG AAC AG- --A
BLY3 --- --- --- -C- --- --- --C -A- -GA --C ---
B43 ACC CTC AAC ATC CAT CCT GTG GAG AAG GTG GAT
25C1 --T --- -CC --- AC- AAC --- C-- TCT AAA --C
BLY3 -G- --- --- --- --- --- A-- --- G-- -AT ---
FR4
B43 TGG ACG TTC GGT GGA GGG ACC AAG CTG GAA ATA
25C1 -AC --- -C- --A --G --- --- --- --- --- ---
BLY3 C-- --- --- --- --- --- --- --- --- --- ---

Fig. 3B-2

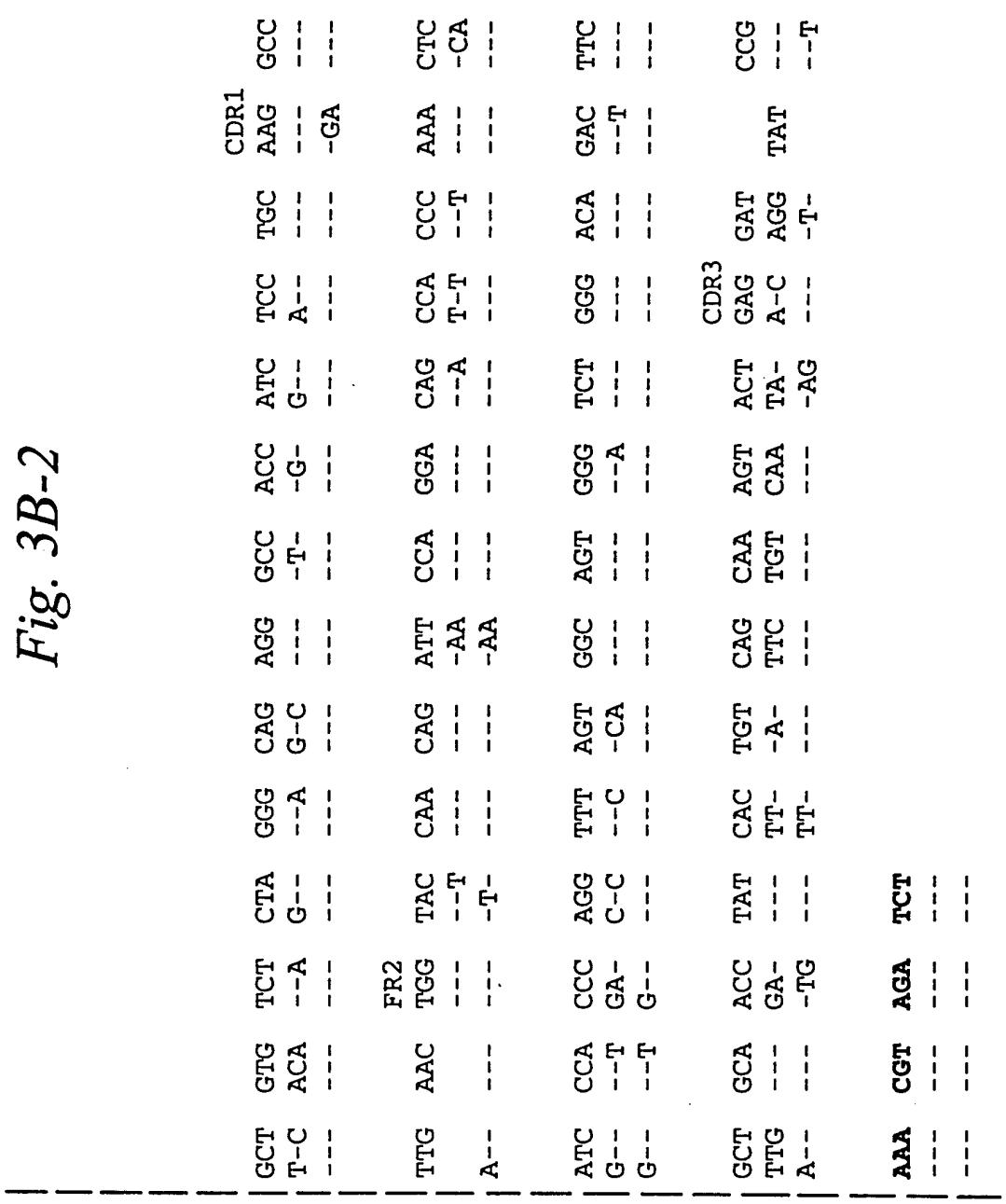

CDR1
FR2
CDR3
GCT GTG TCT CTA GGG CAG AGG GCC ACC ATC TCC TGC AAG GCC
T-C ACA --A G-- --A G-C --- -T- -G- G-- A-- --- --- ---
--- --- --- --- --- --- --- --- --- --- --- -GA ---
TTG AAC TGG TAC CAA CAG ATT CCA GGA CAG CCA CCC AAA CTC
--- --- --T --- --- -AA --- --- --A T-T --T --- -CA
A-- --- --- -T- --- --- -AA --- --- --- --- --- ---
ATC CCA CCC AGG TTT AGT GGC AGT GGG TCT GGG ACA GAC TTC
G-- --T GA- C-C --C -CA --- --- --A --- --- --- --T ---
G-- --T G-- --- --- --- --- --- --- --- --- --- --- ---
GCT GCA ACC TAT CAC TGT CAG CAA AGT ACT GAG GAT CCG
TTG --- GA- --- TT- -A- TTC TGT CAA TA- A-C AGG TAT --- ---
A-- --- -TG --- TT- --- --- --- --- -AG --- -T- --T
AAA CGT AGA TCT
--- --- --- ---
--- --- --- ---

Fig. 3C

A:

	FR1	CDR1	FR2	CDR2
B43	**QVQLLESGA**ELVRPGSSVKISCKASGYAFS	SYWMN	WVKQRPGQGLEWIG	QIWPGDGDTNYNGKFKG
25C1	----------	-----	----------	--Y----------
BLY3	----------A----------	-S---	----------	R-Y----------E

	FR3	CDR3	FR4
B43	KATLTADESSSTAYMQLSSLRSEDSA	VYSCAR	RETTVGRYYYAMDY	**WGQGTTVT**
25C1	Q------K----------G-T-----	------	KTISS-VDF-F --	--------
BLY3	A------K----------T-V---	------	S-YW -N- W----	--------

B:

	FR1	CDR1	FR2	CDR2
B43	**ELVLTQSPA**SLAVSLGQRATISC	KASQSVDYDGDSYLN	WYQQIPGQPPKLLIY	DASNLVS
25C1	---------KFMST-V-D-VSVT-	---N-GTNVA	---K---S--P---	S-TYRN-
BLY3	----------	R------NY-I-FM-	-F--K----------	A---QG-

	FR3	CDR3	FR4
B43	GIPPRFSGSGSGTDFTLNIHPVEKVDAATYHC	QQSTED PWT	FGG**GTKLEIKRRS**
25C1	-V-D--T----------T-TN-QSK-L-D-FY	FCQYNRY-Y-	S----------
BLY3	-V-A----------S-----M-ED-T-M-FC	---K-V -R-	----------

Fig. 4

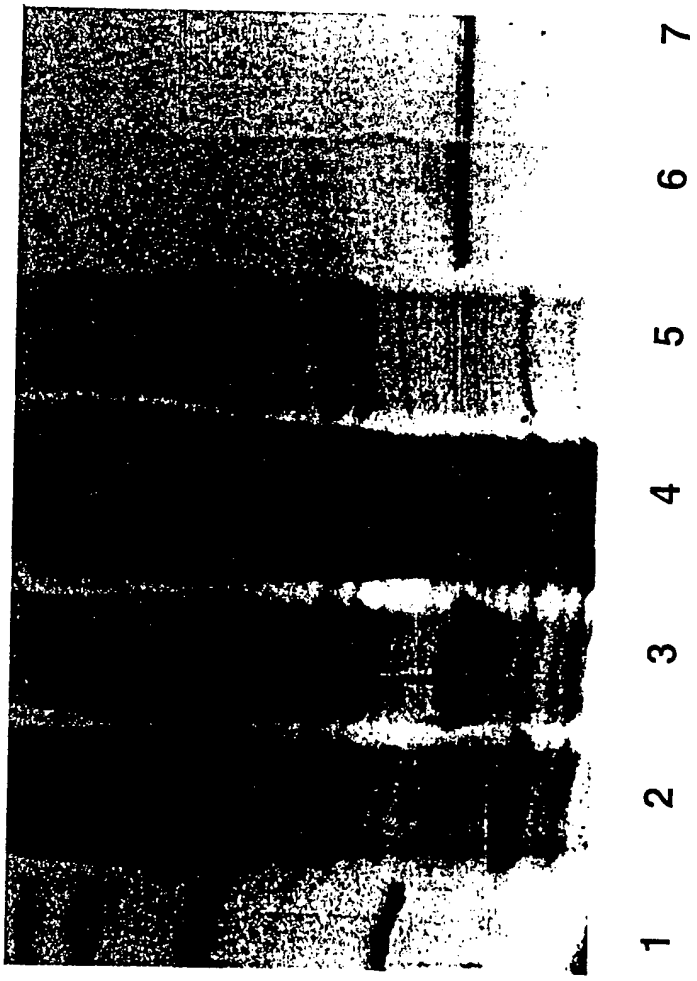


Fig. 5

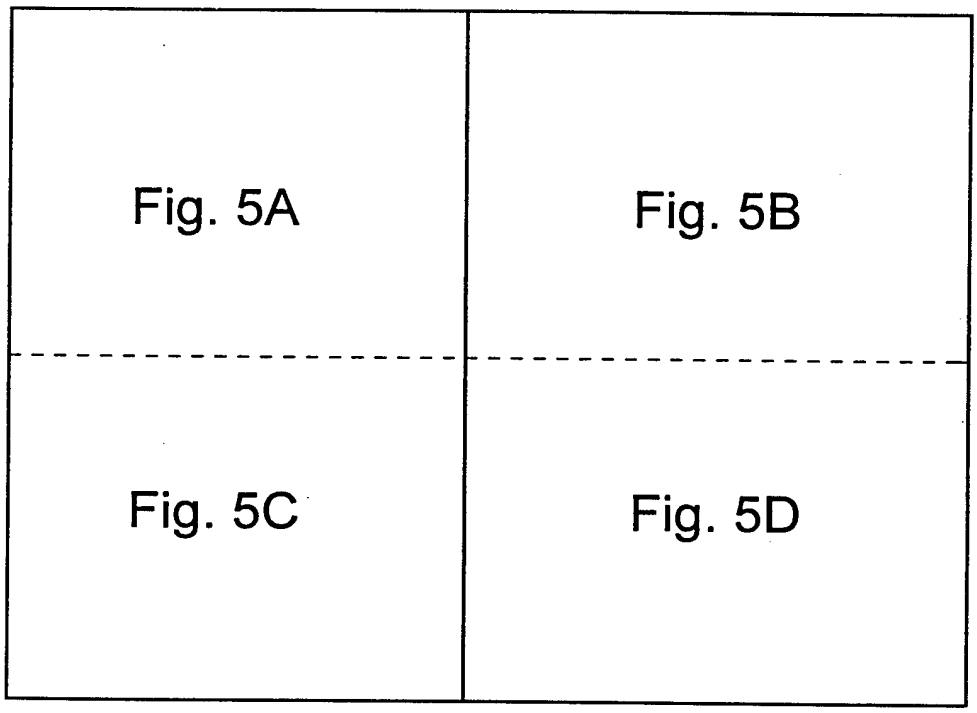


Fig. 5A

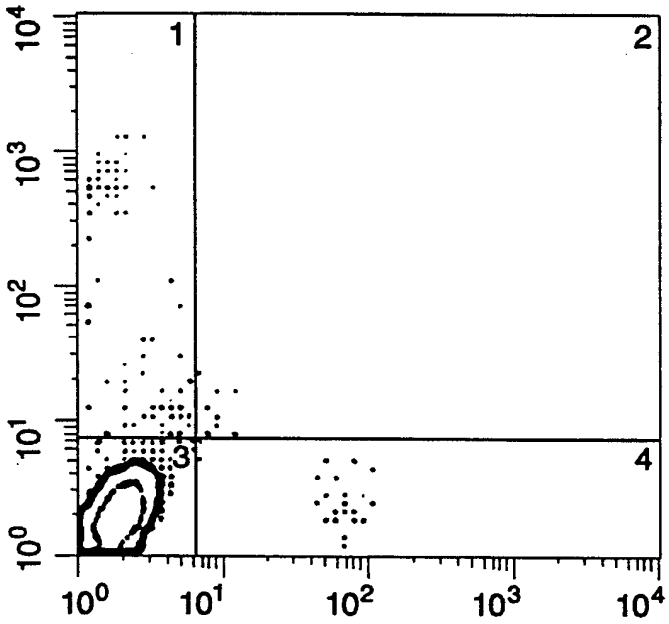


Fig. 5C

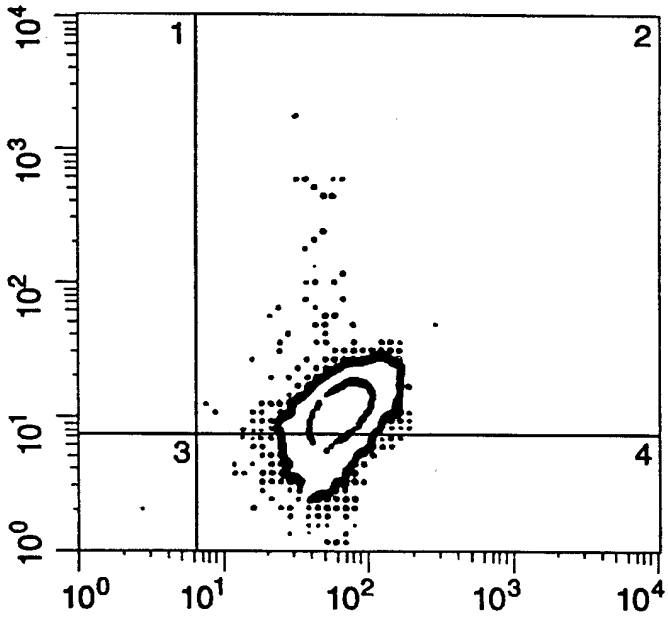


Fig. 5B

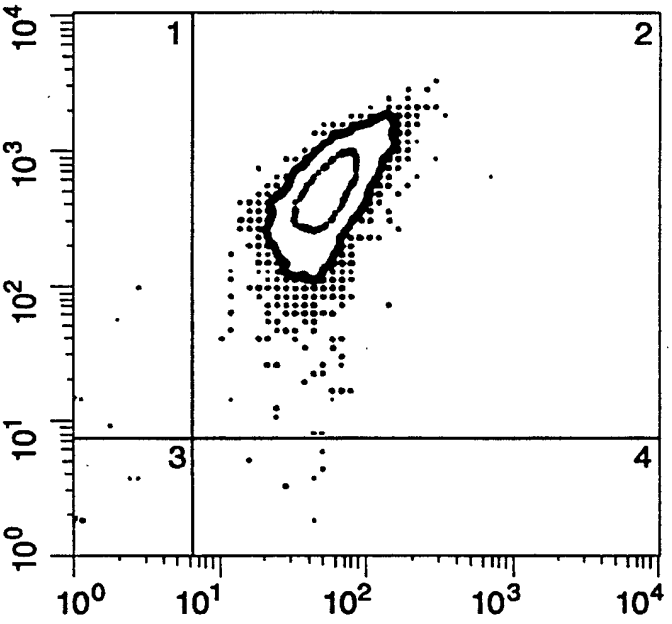
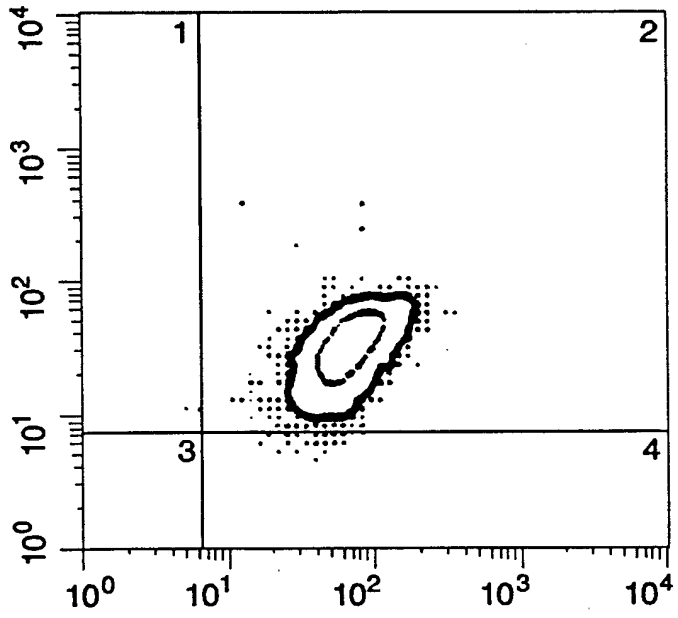


Fig. 5D



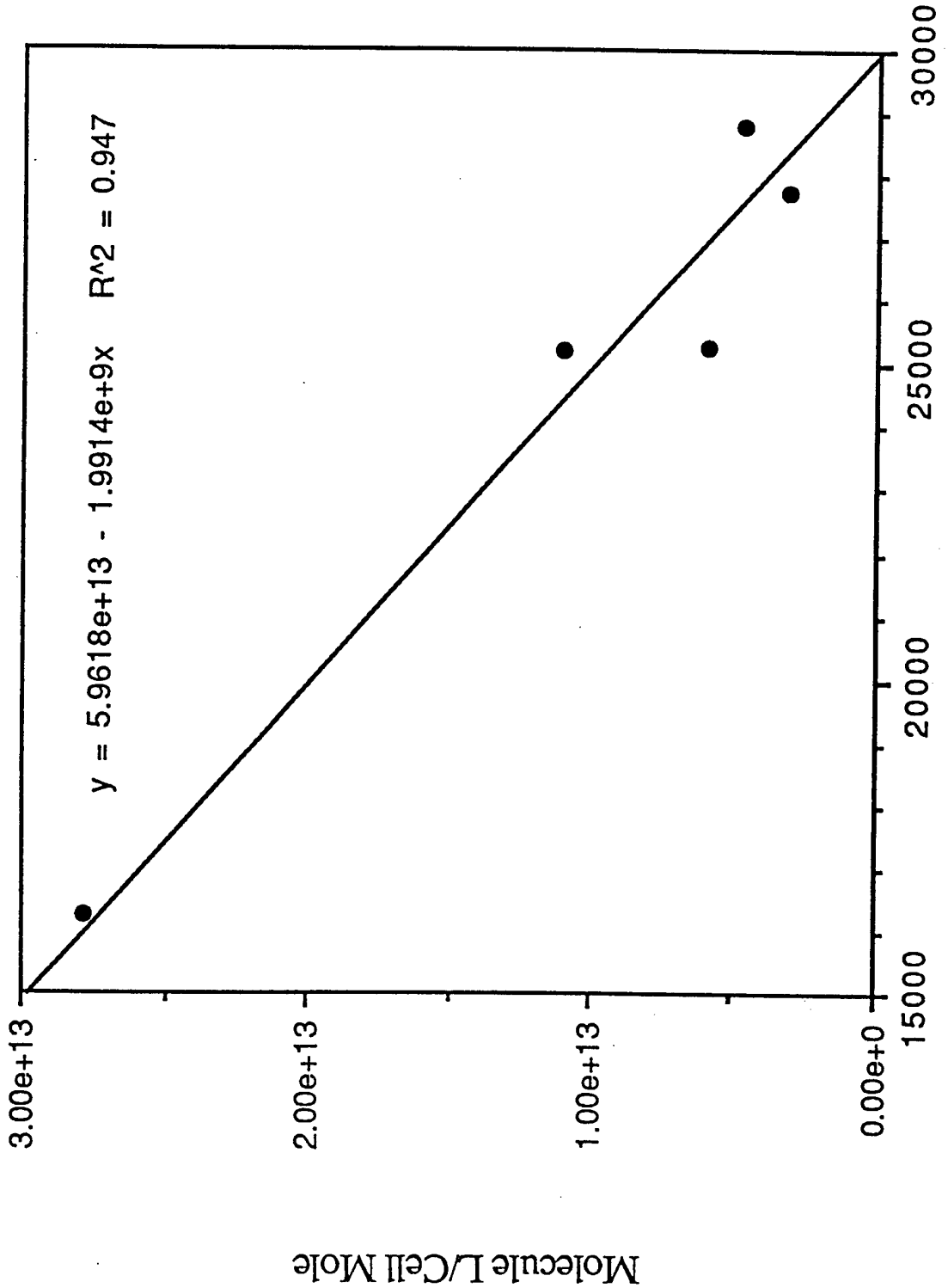

y = 5.9618e+13 - 1.9914e+9x R^2 = 0.947
3.00e+13
2.00e+13
1.00e+13
0.00e+0
15000
20000
25000
30000
Molecule L/Cell Mole
Molecules/Cell

Fig. 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/06941

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 39/395; C07K 16/42; C12N 1/20
US CL :530/387.3, 388.22; 424/134.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/387.3, 388.22; 424/134.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Dialog, CAS, Sequence data bases

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Science, Volume 267, No. 5199, issued 1995, Uckun et al. "Biotherapy of B-Cell Precursor Leukemia by Targeting Genestein to CD19-Associated Tyrosine Kinases", abstract 11573419, see abstract.	1-29
Y	Cancer Reseach, Volume 51, No. 23, part 1, issued 1991, Lambert et al., "An Immunotoxin Prepared with Blocked Ricin A Natural Plant Toxin Adapted for Therapeutic Use", abstract 9057883, see abstract.	1-29

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
23 AUGUST 1996	16 SEP 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer LILA FEISEE Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/06941

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Journal of Immunological Methods, Volume 136, No. 2, issued 1991, Myers et al., "Roduction of Pokeweed Antiviral Protein PAP-containing immunotoxin B43-PAP directed against the CD19 Human B Lineage Lymphoid Differentiation Antigen in Highly Purified Form for Human Clinical Trials", abstract 8177076, see abstract.	1-29
A	Cancer Research, Volume 55, No. 11, issued 1995, Bejcek et al., "Development and characterization of three recombinant dingle chain antibody fragments (scFvs) directed against the CD19 antigen", abstract 11732023, see abstract.	1-29
Y	US,A 5,091,513 (HUSTON ET AL.) 25 February 1992, see entire document.	1-29